

**INTRASPECIFIC VARIATION IN LIFE HISTORY TRAITS OF THE
PANAMANIAN ELECTRIC FISH *BRACHYHYPOPOMUS OCCIDENTALIS***

by

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ABSTRACT

The purpose of this thesis was to investigate intraspecific variation of life history traits in the electric knifefish *Brachyhypopomus occidentalis* under natural conditions, and to explore how individuals optimize their reproduction and brain size under varying predation risks. In the first chapter, I describe the reproductive biology of *B. occidentalis*, using several reproductive traits selected in both females and males to provide insight on the reproductive effort of mature knifefish. I provide field evidence supporting the hypothesis that predation risk environment and geographical isolation drive variation in female reproductive strategies. In the second chapter, I explore whether predation risk and drainage contributed to brain mass variation *B. occidentalis*. I also explore how ontogenetic scaling relationships influence brain mass, and how this association may have been affected by predation. I show that predation risk is an important driver of brain mass variation and discuss the potential implications for the fish and other highly encephalized vertebrates.

Keywords: Life history, knifefish, brain mass, reproduction, expensive tissues.

RÉSUMÉ

Le but de cette thèse était d'étudier la variation intra spécifique des traits d'histoire de vie chez le poisson électrique *Brachyhypopomus occidentalis* sous des conditions naturelles, et d'explorer comment ces individus optimisent leur reproduction et leur masse cérébrale face aux différents risques de prédation. Dans le premier chapitre, je cherche à décrire la biologie de la reproduction de *B. occidentalis*, en étudiant des facteurs de reproduction liés à la valeur sélective (fitness) chez les femelles et les mâles, pour donner un aperçu de l'effort reproductif des poissons matures. Mes résultats suggèrent que le risque de prédation et l'isolement géographique entraînent des changements de stratégies de reproduction chez les femelles. Dans le deuxième chapitre, j'examine si le risque de prédation et l'isolation géographique des bassins contribuent à la variation de la masse cérébrale de *B. occidentalis*. J'explore également comment les relations ontogénétiques influencent la masse cérébrale du poisson électrique, et comment cette association peut être affectée par un haut risque de prédation. Finalement, je discute les implications potentielles de l'impact de facteurs écologiques sur la variation des traits d'histoire de vie chez *B. occidentalis* et chez les vertébrés hautement encéphalisés en général.

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GENERAL INTRODUCTION

Life history theory aims to explain the phenotypic causes of variation in life history fitness correlates, their evolution as adaptive responses to environmental challenges and to identify optimal strategies and potential outcomes (Roff, 1983; Stearns, 1989; Brown and Sibly, 2006; Baulier *et al.*, 2017). Under natural non stressful conditions, traits that optimize fitness, here defined as an organism's ability to produce viable offspring, should be maximized (Johnson and Belk, 2001; Chamberlain *et al.*, 2017). However, environmental constraints such as food availability, optimal temperature, dissolved oxygen availability, predation risk, intra and interspecific competition can modulate the evolution of life history traits, and consequently, limit fitness (Roff *et al.*, 2006; Kotrschal *et al.*, 2015a; Brosset *et al.*, 2016; Karjalainen *et al.*, 2016). Thus, the energetic balance between important competing traits is crucial to maximize organismal fitness (Kozłowski, 1996). Once organisms obtain sufficient energy to ensure basic somatic maintenance (Kotrschal *et al.*, 2019), leftover energy can then be devoted to reproduction (Stearns, 1976; Parker *et al.*, 2018), growth (Rosenfeld *et al.*, 2015), and other costly processes and tissues (Isler and van Schaik, 2006b; Crampton, 2019). Thus, the establishment of life history patterns of a species reflects how natural selection shapes individuals to optimize their survival and reproduction (Stearns, 1989; Karjalainen *et al.*, 2016). In my thesis, I used the electric fish *Brachyhypopomus occidentalis* (Reagan, 1914) as a model to investigate the potential variation in life history traits under natural conditions.

Life history and reproductive traits

Reproduction is one of the most important aspects of a species' biology. Reproductive investments encompass energy allocation to mating systems (Yong and Grober, 2014), gonad

development and maintenance (Roff, 1983; Hayward and Gillooly, 2011), as well as gamete production and mating success (Roff *et al.*, 2006; Parker *et al.*, 2018), to name a few. These investments heavily influence fecundity, offspring quality and quantity, and other life history traits, ultimately impacting fitness (Stearns, 1976; 1989; Baulier *et al.*, 2017). However, reproduction is a costly process for fishes (Jorgensen *et al.*, 2006; Pauly, 2019); likely leading to constraints on the evolution of life history patterns and particularly under environmental stress (Reznick *et al.*, 1992; Billman *et al.*, 2011).

Predation specifically, has been shown to be a major driver of reproductive investment strategies in fishes (Fraser and Gilliam, 1992), with reported changes in fecundity, oocyte mass and age at maturity under varying predation pressures (Travers *et al.*, 2010; Clinchy *et al.*, 2013; Mukherjee *et al.*, 2014; Grégoir *et al.*, 2018). However, the magnitude and direction of change varies within and between species (Magnhagen, 1991; Reznick *et al.*, 2001; Segers and Taborsky, 2012). For example, the introduction of predators is linked to increased reproductive effort in African cyprinid fishes (*Rastrineobola argentea*), with females exhibiting larger oocytes, higher fecundity and clutch volume (Sharpe *et al.*, 2012). Contrastingly, a decline in reproductive effort is reported in threespine stickleback females (*Gasterosteus aculeatus*) in high predation environments, where reduced clutch mass, clutch size and oocyte mass were observed (Heins *et al.*, 2016). Increased predation risk can also lead to adaptive changes in female spawning patterns, including semelparity (death after one reproductive event) and iteroparity (multiple reproductive events in fish lifetime), in response to differences in mortality rates of adults and juveniles (Billman *et al.*, 2011; Albuquerque and Lira, 2016; Lasne *et al.*, 2018). Thus, predation risk can mediate a variety of life history responses to increase either parental or offspring fitness, suggesting that reproduction is a plastic process.

Brain and fitness

Brains are also highly plastic organs, and therefore responsive to environmental stimuli (Striedter, 2005; Kotrschal *et al.*, 2013b; Tsuboi *et al.*, 2014; van der Bijl *et al.*, 2015). Anatomically, the fish brain consists of seven main structures: the telencephalon, the optic tectum, the cerebellum, the dorsal medulla, the pituitary gland, the olfactory bulbs and the hypothalamus, with a number of centres for higher processing homologous to other vertebrate brain regions (Broglia *et al.*, 2003; Ullmann *et al.*, 2010; Bshary and Brown, 2014; White and Brown, 2015). The telencephalon, the foremost brain compartment, is involved in spatial learning, decision making and memory formation in teleosts (Salas *et al.*, 2003; Shumway, 2010; Crispo and Chapman, 2011; Bshary and Brown, 2014; Dunlap *et al.*, 2016). Telencephalon size has also been closely linked to the complexity of the environment, including spatial complexity, habitat structure as well as intra and interspecific interactions (Pollen *et al.*, 2007; Costa *et al.*, 2011). In teleost fish specifically, the telencephalon is involved in conditioned avoidance (Portavella *et al.*, 2004; Portavella and Vargas, 2005), selecting motor actions and evaluating outcomes (Stephenson-Jones *et al.*, 2013; Dunlap *et al.*, 2016). Because of these functions, researchers have often studied the forebrain during predator interactions, as it is thought to be directly linked to predator avoidance mechanisms (Dunlap *et al.*, 2017). In fact, studies on *B. occidentalis* have demonstrated that increased predation risk resulted in decreased forebrain cell proliferation (Dunlap *et al.*, 2016), suggesting that predation risk is a driver for brain size variation in this species.

The brain is also a metabolically expensive organ (Aiello and Wheeler, 1995); thus, the costs of tissue maintenance and development should impose energetic constraints on brain size adaptive evolution (Striedter, 2005; Kotrschal *et al.*, 2013a; Tsuboi *et al.*, 2014; van der Bijl *et al.*, 2015). Across vertebrates, high encephalization (large brain for body size) has been

associated with increased cognitive abilities (Gibson, 2002; Salvanes *et al.*, 2013; Buechel *et al.*, 2018), such as increased spatial learning and numerical learning abilities as observed in guppies, *Poecilia reticulata* (Kotrschal *et al.*, 2013a; b; Kotrschal *et al.*, 2014). Larger brains have also been associated with shifts in fish personality and anti-predator behaviour (Sih *et al.*, 2004; Kotrschal *et al.*, 2013b), as well as decreased susceptibility to predation (van Schaik and Pradhan, 2003; Kotrschal *et al.*, 2015a; Mitchell *et al.*, 2020). Electric fish have been shown to have relatively large brains when compared to other vertebrates (Nilsson, 1996; Kaufman *et al.*, 2003), with some fish having brains that account for up to 3% of their body mass, as opposed to human brains which constitute up to 2.5% (Sukhum *et al.*, 2016). This high encephalization contributes to a large oxygen intake to meet the energetic demands of a larger brain, with some electric fish brains consuming up to 60% of the total oxygen intake (Nilsson, 1996). Thus, the large amount of energy required to develop and maintain brain tissue should impose strong selection against its non-adaptive modification (Striedter, 2005; Tsuboi *et al.*, 2014).

Expensive Tissue and Energy Trade-off Hypotheses

Despite the great degree of plasticity observed in animal brains and reproductive traits, there are costs associated to this plasticity (Aiello and Wheeler, 1995; Striedter, 2005; Tsuboi *et al.*, 2014). Moreover, unlike other somatic tissues, the costs of the brain maintenance and development cannot be temporarily stunted or reduced (Isler and van Schaik, 2009). Particularly in electric fish, the high encephalization along with the associated costs of brain maintenance are likely to impose considerable constraints on brain size evolution (Striedter, 2005; Sukhum *et al.*, 2018). Thus, to compensate for a larger brain, organisms can either increase overall energy intake, or change energy allocation (Isler, 2013). Increased energy intake can occur in a myriad of ways, and fish specifically have been shown to ingest higher

quality food and increase foraging rates (Aiello and Wheeler, 1995; Kotrschal *et al.*, 2015b). However, organisms might not always be in environments with high food availability or quality resources (low food quantity or high inter and intraspecific competition), and increased foraging can be risky under predation threat (Mukherjee *et al.*, 2014), in which case, organisms may resort to allocating resources differently.

Three main theories have been proposed to explain potential shifts in allocation. The “Direct Metabolic Constraints” hypothesis proposes that to mitigate the costs of brain maintenance, organisms should increase their basal metabolic rate (BMR) with increased brain size (Martin, 1981; Hofman, 1983; Tsuboi *et al.*, 2014). The “Expensive Tissue Hypothesis” (ETH) suggests that to compensate for the cost of high encephalization, organisms reduce the size of other metabolically expensive organs (Aiello and Wheeler, 1995; Isler and van Schaik, 2009). Finally, the “Energy Trade-off Hypothesis”, the most recently proposed extension of the ETH (Isler and van Schaik, 2006b), stipulates that any increase in brain size relative to body size is compensated by reduced energy consumption by other plastic and costly functions such as digestion (Isler and van Schaik, 2006b; Tsuboi *et al.*, 2015), locomotion (Navarrete *et al.*, 2011; Tsuboi *et al.*, 2014) or reproduction (Isler, 2013; Kotrschal *et al.*, 2013a; Kotrschal *et al.*, 2015c). Nevertheless, these hypotheses have mainly been tested in mammals and birds (Aiello and Wheeler, 1995; Isler and van Schaik, 2009), and few studies have looked at the ETH or the expensive tissue theory in fishes (Kotrschal *et al.*, 2013a; Kotrschal *et al.*, 2015b). Recent evidence in guppies demonstrates increased brain size in individuals with lower fecundities and smaller gut sizes (Kotrschal *et al.*, 2013a). In this case, intrinsic life history trade-offs would be a direct consequence of competitive allocation of limited resources to one life-history trait versus others, thus imposing constraints on overall fitness (Stearns, 1989; Fraser and Gilliam, 1992; Tsuboi *et al.*, 2015).

***Brachyhypopomus occidentalis* as a model system**

B. occidentalis is a neotropical weakly electric fish of the order Gymnotiformes, family Hypopomidae (Hagedorn, 1988). Gymnotiforms, also known as knifefish because of their compressed body shape, are a prime candidate to study the effects of environmental variation on life history traits, as they inhabit a wide variety of aquatic systems throughout Central and South America (Albert and Crampton, 2003). The genus *Brachyhypopomus* is distributed from Panamá to Argentina (Albert, 2001; Giora *et al.*, 2012), and this geographical range includes diverse freshwater ecosystems such as flooded forests, swamps, floodplains, coastal creeks, as well as forest streams and rivers (Albert and Crampton, 2003; Giora *et al.*, 2012; 2014). In Panamá, *B. occidentalis* primarily inhabit slow-moving shallow rivers and creeks in forests, typically using the floating vegetation and roots as shelter (Hagedorn, 1988). In Panamá, *B. occidentalis* is the only gymnotiform distributed throughout the whole country, and populations can be found in most Atlantic and Pacific drainages (Picq *et al.*, 2014). This provides a study system varying in habitat structure, social complexity and degree of predation risk, to explore how reproductive and life history traits vary in response to environmental variations.

Study question

My thesis focuses on addressing the following question: How do *B. occidentalis* balance reproductive costs as well as the costs associated with brain maintenance, particularly when faced with additional external stressors? Under the energy trade-off hypothesis, multiple predictions can be made resulting in a variety of potential life history strategies that could be displayed in *B. occidentalis* populations. First, brain size may be negatively correlated to expensive organs, following the Expensive Tissue Hypothesis (Aiello and Wheeler, 1995). Secondly, brain size may be inversely linked to the metabolic costs of somatic maintenance

(Isler and van Schaik, 2006b). Thirdly, high encephalization may come at the expense of reproduction, resulting in individuals reducing reproductive effort by producing fewer offspring (low fecundity) or by distributing the costs over a longer developmental period (Isler and van Schaik, 2006b). Little is known about the reproductive biology of *B. occidentalis* (Hagedorn, 1988), and even less is known about the life history strategies of this species. Thus, the purpose of my thesis was to describe the life history patterns of *B. occidentalis* under natural conditions, and to explore how individuals optimize their reproductive output and brain size, particularly under varying predation risks. The main question I aimed to answer in this thesis was whether *B. occidentalis* compensate for large brains by reducing their reproductive output, or whether fish prioritize reproduction under increased predation risk.

Specific objectives

The overall goal of my thesis is to advance our knowledge about the intraspecific variation in life history traits of this electric fish species. Specifically, my main objectives were:

- 1) To determine whether reproductive fitness traits such as gonad size, Fulton's body condition (K), gonadosomatic index (GSI), gonad asymmetry, female absolute and relative fecundity, as well as male sperm morphometry, vary across sites.
- 2) To determine whether brain size variation exists across sites with varying predation risk.
- 3) To assess whether there are energy trade-offs between expensive tissues such as the brain and gonads.
- 4) If trade-offs exist between reproductive traits and brain size, to assess whether these trade-offs vary based on predation risk or drainage.

Hypotheses and predictions

For Chapter I, I hypothesized that predation risk induces differences in the reproductive traits of natural populations of *B. occidentalis*. Specifically, I predicted that:

- 1) Because female reproduction is costly, I expect that female reproductive traits such as oocyte diameter, volume, relative fecundity (number of mature oocytes per gram of body mass) and absolute fecundity (total number of oocytes per female) should vary across populations. I expect females from high predation risk sites to have smaller ovaries, lower absolute and relative fecundity, along with smaller oocyte traits.
- 2) Sperm morphology traits such as sperm total length, head length, head width and flagellum length should vary across populations. I expect males at high predation risk sites to have smaller testes and have spermatozoa that have shorter tails and smaller heads.

For Chapter II, I hypothesized that predation risk induces variation in overall brain mass in natural populations of *B. occidentalis*. Specifically, I predicted that:

- 1) Brain mass would vary among sampling sites and river drainages, likely associated with variation in predation risk.
- 2) If differences in brain mass are observed among drainages and sites, this same variation will be observed in brain-body ontogenetic allometry, with population differences being linked to variation in predation risk.

CHAPTER I: Reproductive biology of *Brachyhypopomus occidentalis*

ABSTRACT

The reproductive fitness of organisms often depends on various environmental factors, including temperature, food availability, geographical isolation and predation. Changes in the optimal range of factors can be perceived as stressors, forcing organisms to optimize energy investment, creating trade-offs. In this chapter, I describe the reproductive biology of the electric knifefish *Brachyhyopomus occidentalis* under natural conditions. I also investigate whether predation risk or drainage contribute to variation in reproductive output. I sampled fish from high and low predation risk sites within two geographically isolated river drainages in eastern and central Panamá. Gonad mass, gonad asymmetry and gonadosomatic index were analysed in both females and males, along with oocyte and sperm morphology traits, to provide insight on the reproductive fitness of mature *B. occidentalis*. Overall, I observed sexual dimorphism, with males being larger and heavier than females. Fish from low predation risk sites were significantly larger, with heavier gonads and higher gonadosomatic indices. Females under low predation risk invested highly in gonad development. I observed larger ovaries, higher relative and absolute fecundities, as well as higher oocyte quantities per maturity stage. I also observed a higher prevalence of gonad asymmetry in low predation risk sites. I also observed drainage differences, where females from the central Drainage (Chagres) exhibited heavier ovaries and greater GSIs, along with higher absolute and relative fecundities, mature oocyte quantity and oocyte traits. For male *B. occidentalis*, I observed no difference in testes traits or sperm morphological traits based on site, predation risk or drainage. Collectively, my results reveal a clear association between predation risk, geographical isolation, and female reproductive strategies.

INTRODUCTION

Reproduction is a fundamental aspect of a species' biology, and it is an important driver of fitness of individuals. Reproductive fitness, the ability of an individual to produce viable offspring, can be determined by a multitude of factors, including variations in morphological, behavioural, physical and physiological traits (Anholt *et al.*, 2020). In fish, the cost of reproduction is assumed to be higher in females than males, because females tend to prioritize and maximize offspring survival, whereas males' priority is to inseminate as many females as possible (Parker and Pizzari, 2010; Hayward and Gillooly, 2011). As a result, correlates of reproductive fitness in fishes may differ between males and females and across species. In females specifically, gonad development and maintenance, as well as egg quantity and quality have been used as proxies for reproductive fitness (Hayward and Gillooly, 2011; Arantes *et al.*, 2013; Giora *et al.*, 2014; Baulier *et al.*, 2017); while in male fishes, sperm production, sperm morphometry and sperm swimming performance are used as proxies of individual reproductive success (Stockley *et al.*, 1997; Simpson *et al.*, 2014; Martinez *et al.*, 2015).

Yet, for both males and females, reproduction can be energetically costly (Roff, 1983; Moore *et al.*, 2016; Chamberlain *et al.*, 2017). In many cases, mature individuals must trade off the proportion of resources allocated to reproduction against other life-history traits such as growth (Roff, 1983; Brosset *et al.*, 2016), and survival (Magnhagen, 1991; Baulier *et al.*, 2017), and these trade-offs are likely influenced by the external environment (Reznick *et al.*, 2001; Billman *et al.*, 2011; Su *et al.*, 2020). For instance, variation in environmental factors, such as predation pressure or limited resources (Johansson *et al.*, 2018; Devigili *et al.*, 2019) can lead to a physiological stress response in fishes (Barton, 2002; Hawlena and Schmitz, 2010), resulting in changes in behaviour and allocation of resources to processes that maximize

survival rather than reproduction (Clinchy *et al.*, 2013; Johansson *et al.*, 2018; Devigili *et al.*, 2019). Another aspect likely influencing reproductive biology is fluctuating asymmetry, defined as the random deviation of bilaterally symmetrical traits (Urbach *et al.*, 2007). Fluctuating asymmetry has long been regarded as a proxy of an individual's ability to cope with genetic and environmental stressors (Palmer and Strobeck, 1986; Moller, 1994; De Coster *et al.*, 2013). Because bilateral traits generally develop under one identical genome (Møller and Pomiankowski, 1993b; De Coster *et al.*, 2013), the random fluctuations between the right and left side of normally symmetrical organs is assumed to reflect the organism's inability to buffer their development against perturbations, also known as developmental instability (Møller and Pomiankowski, 1993a; Groenendijk, 1998; Urbach *et al.*, 2007). Studies have shown that traits under sexual selection, such as gonad mass, show higher rates of fluctuating asymmetry (Møller, 1993; Polak and Taylor, 2007; De Coster *et al.*, 2013); and in fishes, there are reports of negative correlations between fluctuating asymmetry and offspring survival (Wedekind and Mueller, 2004), increased mate competition (Burness *et al.*, 2008) and mate attractiveness (Gross *et al.*, 2007; Polak and Taylor, 2007), making fluctuating asymmetry a useful tool when quantifying an organism's reproductive fitness.

The aim of my study was to investigate the reproductive biology of the Panamanian knifefish *Brachyhypopomus occidentalis* (Regan, 1914), a gymnotiform weakly electric fish found in South American freshwaters, expanding to Central America and up to Panamá (Picq *et al.*, 2014). Gymnotiforms make an excellent system to study the variation of reproductive traits, particularly in terms of the effects of sexual selection on the variation of important fitness traits (Crampton, 2019). For instance, there is observable sexual size dimorphism across the *Brachyhypopomus* genus (Hopkins, 1999; Curtis and Stoddard, 2003; Waddell *et al.*, 2019), where fish exhibit seasonal sexual dimorphism in caudal filament length (Waddell *et al.*, 2019).

In *B. occidentalis*, the electric organ is situated along the body axis to the tip of the caudal filament (Tran, 2014), resulting in observable differences in the generation, duration and strength of electric organ discharges (EODs) between males and females (Hagedorn and Carr, 1985). The sexual dimorphism in signal production suggests that there are differential costs between the sexes (Hopkins, 1999; Salazar and Stoddard, 2008). Thus, a variation in life history trade-offs should be observed.

Although the electric system used by this species has been extensively investigated (Hagedorn and Carr, 1985; Stoddard, 1999; Stoddard and Markham, 2008; Dunlap *et al.*, 2016; Picq *et al.*, 2016; Stoddard *et al.*, 2019), the reproductive biology of this species remains understudied (Hagedorn, 1988). In this chapter, I described reproductive traits of natural populations of *B. occidentalis* within two isolated drainage systems in Panamá, that have been previously used as study systems (Tran, 2014; Dunlap *et al.*, 2016; Picq *et al.*, 2016). I also investigated whether reproductive traits vary in this species based on predation risk. Because fish reproductive traits have been previously linked to variations in ecological factors (Johnson and Belk, 2001; Devigili *et al.*, 2019), and the studied populations are genetically distinct (Picq *et al.*, 2014; Picq *et al.*, 2016), I hypothesized that predation risk has the potential to induce differences in the reproductive traits of natural populations of *B. occidentalis*. To address this issue, I measured the following reproductive parameters: gonad mass, gonadosomatic index, gonad asymmetry, absolute and relative fecundity, oocyte maturity, oocyte size as well as sperm size. My predictions for this chapter were the following:

- 1) There will be sexual dimorphism of general morphological traits, where males will be longer and heavier than females independently of site, predation risk and drainage.
- 2) Reproductive traits will vary between sites facing different predation risks. Fish from low predation risk sites will invest more in gonad development.

- 3) Female reproductive traits such as ovary mass, relative fecundity (number of mature oocytes per gram of body weight), absolute fecundity (total number of oocytes per female), and specific oocyte traits (oocyte diameter, volume and surface area) will vary across sites and based on predation risk and drainage.
- 4) Sperm morphology traits such as sperm total length, head length, head width and flagellum length will vary across sites, and based on predation risk and drainage.

MATERIALS AND METHODS

Sampling sites and fish capture

I sampled four sites located within two geographically isolated river drainages (Charges and Bayano) in central and eastern Panama (Figure 1.1). These sites were selected to reflect differing predation pressures (Table 1.1), based on prior research (Picq *et al.*, 2014; Dunlap *et al.*, 2016; Stoddard *et al.*, 2019).

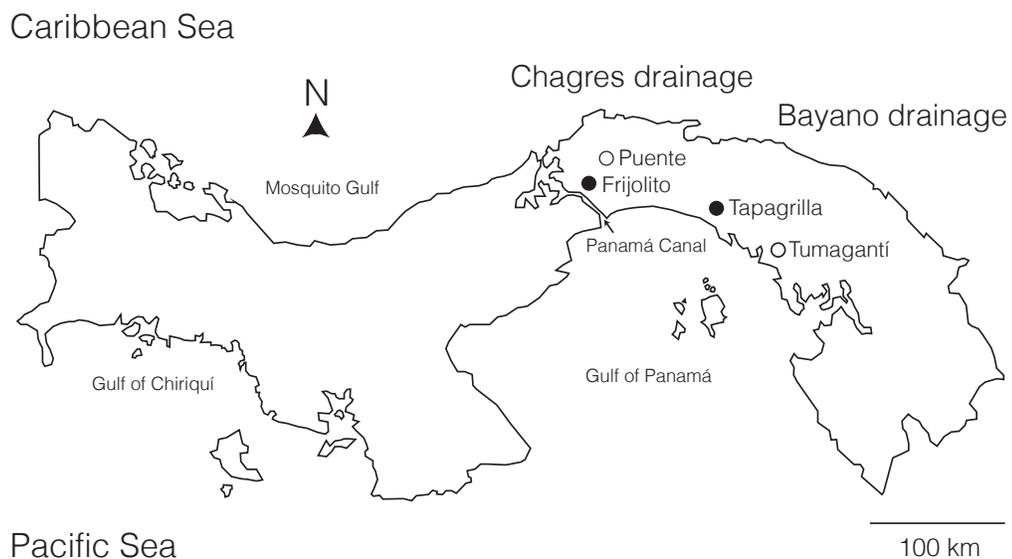


Figure 1.1. Map of the Republic of Panama showing the four sampling sites within the two isolated drainages. Open circles indicate the populations with high predation pressure whilst filled circles indicate the populations with low predation pressure.

Specifically, high predation risk sites have a high density of predatory catfish, *Rhamdia quelen* (Picq *et al.*, 2014; Dunlap *et al.*, 2016). Exposure to *R. quelen* often results in fish tail injury, where tails are partially or completely bitten off (Tran, 2014); thus, indicating predation by catfish. In this study, I considered both presence of predators and incidence of tail injury reported in *B. occidentalis* to determine which sites were of high predation risk (Appendix 1).

Table 1.1. Sampling sites in the Chagres and Bayano Region in Panamá.

Site	Type	Predation risk
Bayano (eastern Panama)		
Tapagrilla (<i>n</i> =22)	Stream	High
Tumagantí (<i>n</i> =14)	River	Low
Chagres (central Panama)		
Frijolito (<i>n</i> =25)	Stream	High
Puente (<i>n</i> =21)	Stream	Low

Note: number of individuals captured per site (*n*). Coordinates were as follows: Frijolito (09°08'0.38" N, 79°43'28.9" W), Puente (09°10'8.88" N, 79°31'28.9" W), Tapagrilla (09°09'0.2.5" N, 79°10'47.2" W) and Tumagantí (09°13'5.40" N, 78°53'36.5" W). Predation risk based on incidence of tail injury (Appendix 1) and predator density (Tran, 2014).

Frijolito, Tapagrilla and Puente were fairly similar streams, with clear water, low current, and dense and mature canopy overhead (Figure 1.2). These streams were less than 5 m wide, with stream beds armoured with rocks, and depths ranging anywhere from 10 cm to 1.5 m. These locations are ideal for the nocturnal *B. occidentalis* as the leaf beds, tree roots and floating debris made for ideal hiding spots (Hagedorn, 1988). Tumagantí, however, differed from the other sites in its depth, width and current speed, the riverbed consisted primarily of varying boulder sizes, and mature forests in its surroundings. This site had a strong water current, and the roots of trees were more abundant and exposed at the riverbank level, offering the fish a greater protection, and at the same time making it harder to capture the fish.

Brachyhypopomus occidentalis coexists with a wide variety of fish species such as *Astyanax* sp., *Panamius panamensis*, *Rineloricaria* sp., and *Brachyrhaphis* sp., other knifefish such as *Sternopygus dariensis* and *Apternotus rostratus*, as well as the native predator *Rhamdia quelen* (personal observation).



Figure 1.2. Sampling sites in Panamá, where *Brachyhypopomus occidentalis* were collected. Puente, in the Chagres drainage (left), Tumagantí, in the Bayano drainage (right).

In total, I collected 82 fish in mid-January 2018 from all sites. From all the collected fish, 65 displayed signs of reproductive maturity. Fish were electrically located using an audio mini amplifier (Radioshack®) connected to electrodes on a plastic rod (fish locator). The fish locator was completely submerged into the stream water allowing for an audible signal transduction of the electric organ discharge (EOD) through the mini amplifier. Once the fish finder was near an electric fish, the amplifier would pick up the fish EOD, allowing for a precise location. After refining the exact location of the fish, generally under root masses, between rocks or under leaf litter (personal observation), fish were captured using a 30 x 40 cm rectangular dip net. Following capture, fish were individually placed into a labeled minnow bag filled with stream

water, and with enough air to allow transportation to the lab at the Institute for Scientific Research and High Technology Services of Panama (INDICASAT-AIP) for processing.

Fish morphometrics

I euthanized the fish using a hypothermic shock in an ice bath (scientific permit #SE/AB-1-17 MIAMBIENTE). Fish were then placed on their right side on a neutral grey photo-corrected board fitted with a ruler, and a white label with the fish ID number. At least five pictures per fish were taken using a Canon PowerShot G5 X. Photos were taken perpendicularly and levelled to the specimen. Photos were used for morphology analyses. Body mass was obtained using a pro pocket SmartWeigh® scale. Body morphology traits obtained from each fish included: total fish length (± 0.1 cm) and body mass (± 0.01 g). Using these two traits, Fulton's condition factor (Neff and Cargnelli, 2004) was calculated as follows:

$$K = \left(\frac{M_s}{L_T^3} \right) \times 100$$

where M_s is somatic mass (body mass minus gonad mass), L_T is total body length. The Ratio Index (RI) of the fish was also calculated to account for the elongated shape of the fish as described by Falk *et al.* (2017) using the same two parameters as follows:

$$RI = \left(\frac{T_M}{L_T} \right)$$

where T_M is the total body mass and L_T is total body length.

Gonad analyses

I carefully dissected the gonads from the abdominal cavity, and their wet mass was taken using the SmartWeigh® High Precision Milligram Jewelry Scale GEM50 for the right and left gonad

individually (± 0.0001 g). Using the total gonad mass, I calculated the gonadosomatic index, GSI (Giora *et al.*, 2014), as follows:

$$GSI = \left(\frac{G_T}{M_S} \right) \times 100$$

where G_T is the total gonad weight, and M_S is the somatic mass. Gonad asymmetry (GA) was calculated by subtracting the right gonad mass from left gonad mass, such that a negative value indicates a larger right gonad, while a positive result indicates a larger left gonad (Perrault, 2015).

Ovary and oocyte analysis

Ovaries were immediately preserved in 95% buffered ethanol to avoid shrinkage until processing. I determined ovary maturity based on macroscopic observations (Chang *et al.*, 2018). Briefly, oocytes were assigned to four different maturity levels: immature, developing, maturing and mature (Table 1.2).

Table 1.2. Macroscopic description of ovary maturity stages in *Brachyhypopomus occidentalis*.

Maturity stage	Ovary Traits
Immature	Off white in colour, very small ($G_w < 0.04$ g), difficulty distinguishing gonads
Maturing	Beige and opaque, small in size ($G_w < 0.17$ g), oocytes visible, no mature or maturing oocytes present
Mature	Light yellow and opaque, firm to the touch, blood vessels are visible and ovary membrane is very thin, oocytes from all maturity stages are present
Spent	Bright yellow/ orange, multiple blood vessels, $G_w > 0.032$ g, high proportion of mature oocytes

Note: This table was created based on the Macroscopic characters of gonad maturity stages (Waddell and Crampton, 2018). G_w indicates ovary mass.

I dissected the oocytes from the ovary membrane and placed them on a neutral grey photo-corrected board fitted with a ruler, and a white label with the corresponding fish ID number. Every individual oocyte from each ovary was counted four times to calculate the average absolute fecundity (AF), or total number of eggs per female (Giora *et al.*, 2012), while the relative fecundity (RF) was calculated as follows:

$$RF = \left(\frac{M_{ooc}}{M_T} \right)$$

where M_{ooc} is total the number of oocytes and M_T is the total body weight of the female (Giora *et al.*, 2014).

Once the oocytes were separated by maturity level (Table 1.3), I haphazardly selected 20 individual eggs per maturity level per fish to measure their volume and surface area. I took photos of the different egg stages per female using an Infinity 1 digital camera (Lumenera Corporation, Ottawa, Canada) attached to a Leica stereoscope (Leica Microsystems, Wetzler, Germany). I calculated oocyte diameter ($\pm 0.01 \mu\text{m}$), volume ($\pm 0.01 \mu\text{m}^3$) and surface area ($\pm 0.01 \mu\text{m}^2$) using the Egg Tool extension (Troscianko, 2014) in the Image J software (NIH 1.52; see Appendix 2). The oocytes were preserved in 95% ethanol.

Table 1.3. Macroscopic description of oocyte maturity stages in *Brachyhypopomus occidentalis*.

Maturity stage	Oocyte
Immature	Off white/beige, very small in diameter, very fragile
Maturing	Light yellow or beige, small size but firm
Mature	Yellow/orange in color, opaque, hard, large in diameter and volume

Testes and sperm analysis

I determined testes maturity following a standard protocol (Uribe *et al.*, 2014). Based on macroscopic observations, I classified testes within three categories (Table 1.4). Sperm morphology analysis was done by extracting 4 μ l of milt from the punctured testes diluted into 200 μ l of 10% formalin (Fisher Scientific, Ottawa, Canada), and transferring it to a microscope slide. The slides were tilted at a 45° angle and left to air dry for 24 h. Testes were preserved in 95% ethanol. Slide staining and sperm morphology analysis were done according to the method described by Perrault (2015). Briefly, staining of slides was done by immersion into full strength methanol for 10 min, and subsequent immersion in Eosin Y for 7 min, followed by a final rinse with distilled water. Slides were allowed to dry overnight before being sealed using Permount® (Fisher Scientific, Nepean, Ontario, Canada).

Table 1.4. Macroscopic description of testicle maturity stages in *Brachyhypopomus occidentalis*.

Maturity stage	Testes Traits
Immature	Translucent, very small, difficulty distinguishing gonads
Maturing	Translucent white, small ($G_w < 0.020$ g), vascularization becomes apparent
Mature	Cream and opaque, gelatinous to the touch, blood vessels are present, bigger than Maturing stage ($G_w > 0.020$ g).

Note: This table was created based on the Testes Maturity Index (Uribe *et al.*, 2014). G_w indicates testes mass.

Using an Olympus CX41 light microscope (Olympus Scientific Solutions Americas, Waltham, Massachusetts, USA) and the Infinity 1 digital camera, I took pictures of the sperm smears for sperm morphometry analyses. For each specimen, I photographed a minimum of 20 individual spermatozoa per fish and took the following measurements using Image J software:

1. H_L : the length of the spermatozoa head from flagellum insertion point to the anterior tip of the head ($\pm 0.01 \mu\text{m}$),
2. H_W : the width of the spermatozoa head perpendicular to length and from the widest point of the head ($\pm 0.01 \mu\text{m}$),
3. F_L : the length of the spermatozoa flagellum from the insertion point of the sperm head to the tip of the tail ($\pm 0.01 \mu\text{m}$).

With these measurements, I calculated the total sperm length by adding the average head length to the average flagellum length. I also calculated the hydrodynamic ratio, HR (head length to head width) and head length to flagellum length ratio (Humphries *et al.*, 2008a) as follows:

$$HR = \frac{H_W}{H_L}$$

where HR is the hydrodynamic ratio, H_W is the head width, H_L is the head length (μm); and

$$HL:FL = \left(\frac{H_L}{F_L} \right)$$

where $HL:FL$ is the head length to flagellum length ratio, H_L is the head length, and F_L is the flagellum length (μm).

STATISTICAL ANALYSES

Data were analyzed using IBM SPSS Statistics 24. For the purpose of this chapter, I only used data from mature fish. All data were natural log transformed to meet assumptions of normality and heterogeneity of variance. Assumption of normality was verified using the residuals of the Q-Q plots for each variable. I started by testing for evidence of sexual dimorphism in the following morphological traits: total length (T_L), somatic mass (S_M) and Fulton's condition (K) by performing a 2x2x2 factorial ANOVA using sex, drainage and predation as the main effects.

I then conducted 2x2 factorial ANOVAs followed by Tukey's post-hoc tests for each sex separately to further explore the main effects of drainage and predation risk on the selected morphological traits. Because reproductive traits vary greatly between males and females, and depending on the size of the fish, I explored the variation of reproductive traits based on mass and length. In all cases, length had a greater effect, and thus, I used length as a covariate in the remainder of the analyses. Using a 2x2 factorial ANCOVA, I examined the main effects of predation risk and drainage on gonad mass (G_w), gonadosomatic index (GSI) and gonad asymmetry (GA) for each sex. Fish from the site Tapagrilla were omitted from the reproductive trait analyses because for females, all the observed ovaries were either atrophied or too small to distinguish oocytes, and for males, I was not able to extract milt from any of the testes. As a result, the effects of predation risk and drainage on oocyte diameter (D), oocyte volume (V) and oocyte surface area (SA) were analyzed separately using a one-way ANOVA for each oocyte category. I also tested the main effects of site, predation risk and drainage on absolute and relative fecundity by conducting the same analysis. For sperm trait analyses, I tested the main effects of site, predation risk and drainage on sperm length, head width, head length, flagellum length, hydrodynamic ratio (HR) and head to flagellum length percentage (HL:FL) using one-way ANCOVAs followed by Tukey post-hoc tests. Samples were considered significantly different when $P < 0.05$.

RESULTS

Morphological trait variation

My results reveal that morphological traits vary significantly based on sex and predation risk (Table 1.5). Male *B. occidentalis* were 14.4% longer and 47.6% heavier than females (Figure 1.3). Overall, fish from high predation risk sites were 9.5% shorter, 14.2% lighter and

had K values that were 36.6% lower than fish from low predation risk sites. When analyzing total length within the high predation risk populations, I observed significant differences based on drainage ($F_{1,29} = 20.8$; $P < 0.0001$), where Chagres fish (Frijolito) were 16.1% longer than Bayano fish (Tapagrilla). I observed no significant effect of sex in high predation risk populations ($P = 0.38$); however, I did observe a significant interaction between sex and drainage ($F_{1,29} = 6.27$; $P = 0.021$). Further analysis reveals that within Chagres populations, male fish at the high predation risk site (Frijolito) were 14.7% longer ($F_{1,15} = 7.40$; $P = 0.017$), while there was no observable difference in Bayano ($P = 0.30$). For the low predation risk populations, I observed a significant difference in total length based on sex ($F_{1,34} = 7.03$; $P = 0.013$), with male fish being about 12.6% longer than females. Drainage had no significant effect on low predation risk total length variation ($P = 0.22$), and I observed no interaction between sex and drainage ($P = 0.09$).

Table 1.5. Results of the 2x2x2 Factorial Analysis of Variance (ANOVAs) examining the main effects of sex, predation and drainage on total length (T_L), somatic mass (S_M) and Fulton's condition factor (K) of *B. occidentalis* in Panamá ($n= 65$).

Effect	d.f.	T_L		S_M		K	
		F	P	F	P	F	P
Sex	1	5.99	0.02*	8.64	0.005*	0.02	0.90
Predation	1	9.91	0.003*	4.64	0.04*	5.79	0.02*
Drainage	1	2.44	0.12	1.22	0.27	1.03	0.32
Sex x Predation	1	2.13	0.15	4.20	0.045*	0.20	0.65
Sex x Drainage	1	0.004	0.95	0.05	0.82	0.53	0.47
Predation x Drainage	1	11.7	0.001*	13.2	<0.0001*	0.39	0.53
Sex x Predation x Drainage	1	6.86	0.01*	7.56	0.008*	0.15	0.70
Error		57		57		57	

Note: Descriptive variables are as follows: total length (T_L), somatic mass (S_M) and Fulton's condition (K). Asterisks show a significant effect of predation on observed morphological trait ($P < 0.05$).

Drainage also had a significant effect on somatic mass variation in high predation risk populations ($F_{1,29} = 12.2$; $P = 0.002$). Fish in Chagres were on average 42.6% heavier than fish from Bayano. I observed no significant effect of sex on somatic mass ($P = 0.52$) nor any interaction between sex and drainage ($P = 0.07$) in high predation risk populations. When looking at low predation risk populations, drainage had no significant effect on somatic mass variation ($P = 0.07$). However, male *B. occidentalis* were about 53.8% heavier than females at low predation risk sites ($F_{1,29} = 13.6$; $P < 0.0001$); and there was a significant interaction between drainage and sex ($F_{1,34} = 4.85$; $P = 0.035$). Further analysis revealed that within Bayano, males were 107% heavier than females ($F_{1,13} = 16.9$; $P = 0.001$), while in Chagres somatic mass did not differ between sexes ($P = 0.27$).

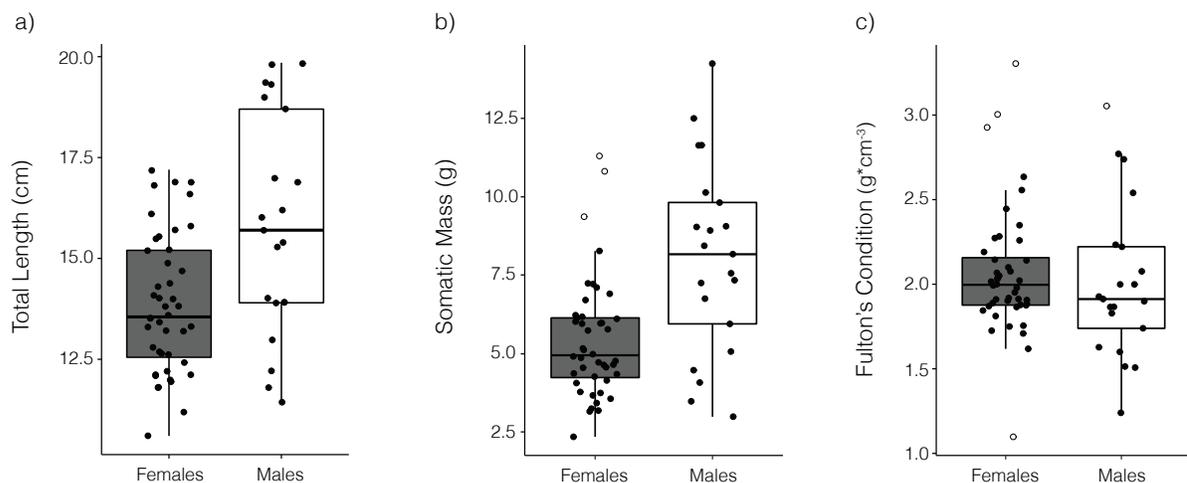


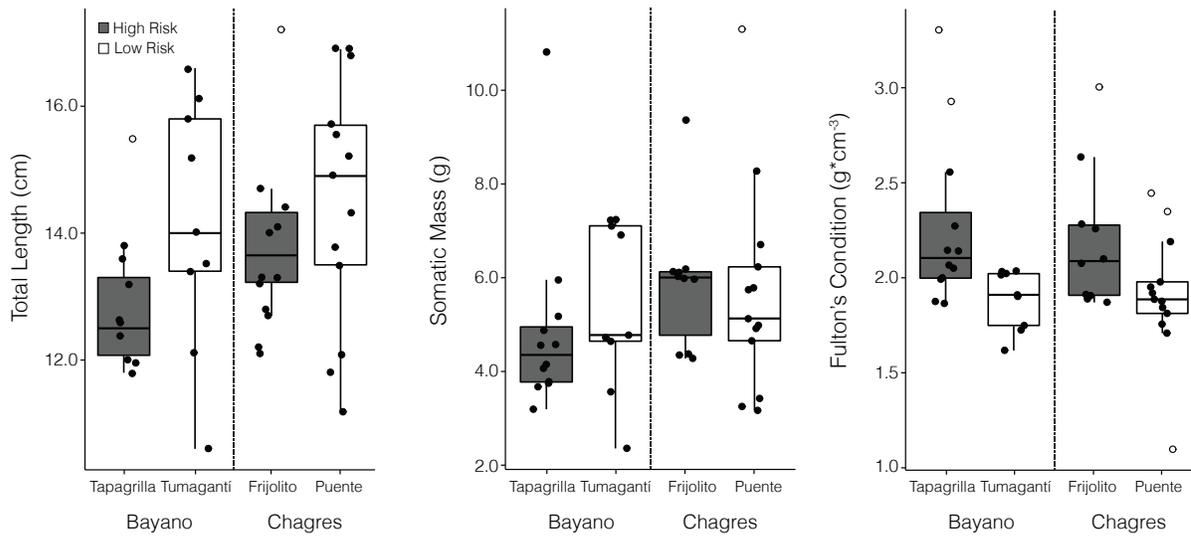
Figure 1.3. Sex-based variation in a) total length b) somatic mass and c) Fulton's condition factor of sampled *B. occidentalis* populations. The dark gray boxplots represent females ($n = 44$ fish) whilst the white boxplots represent males ($n = 21$ fish). The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values respectively, while the black dots indicate individual values. The open dots represent outliers.

Body morphology variation based on sex

When analyzing the sexes separately, Fulton's condition of females varied based on predation risk ($F_{1,43} = 9.70$, $P = 0.003$), with females from low predation risk having a lower K value than females from the high predation risk sites (Figure 1.4a). Female total length and somatic mass did not vary based on predation risk ($P = 0.09$, $P = 0.92$ respectively). My results also show no significant variation in total length ($P = 0.12$), somatic mass ($P = 0.21$) and K ($P = 0.75$) based on drainage in females.

In males, I observed significant differences between high predation risk and low predation risk populations in two body traits (Figure 1.4b). Specifically, male total length ($F_{1,20} = 5.31$, $P = 0.034$) and somatic mass ($F_{1,20} = 6.03$, $P = 0.025$) varied based on predation risk, while no difference was observed in K ($P = 0.37$). Furthermore, I observed no significant variation in total length ($P = 0.43$), somatic mass ($P = 0.62$) or K ($P = 0.42$) based on drainage in males. I observed significant interactions between predation risk and drainage for total length ($F_{1,20} = 9.12$, $P = 0.008$), and somatic mass ($F_{1,20} = 13.9$, $P = 0.002$). Further analysis shows that in Bayano, high predation risk males were significantly shorter than low predation risk males ($F_{1,6} = 11.9$, $P = 0.018$); while in Chagres, no difference was observed in male length ($P = 0.54$). Similarly, Bayano males from the high predation risk site were significantly lighter than males from the low predation risk site ($F_{1,6} = 37.3$, $P = 0.002$); while no significant difference in mass was observed in Chagres ($P = 0.32$).

a) Females



b) Males

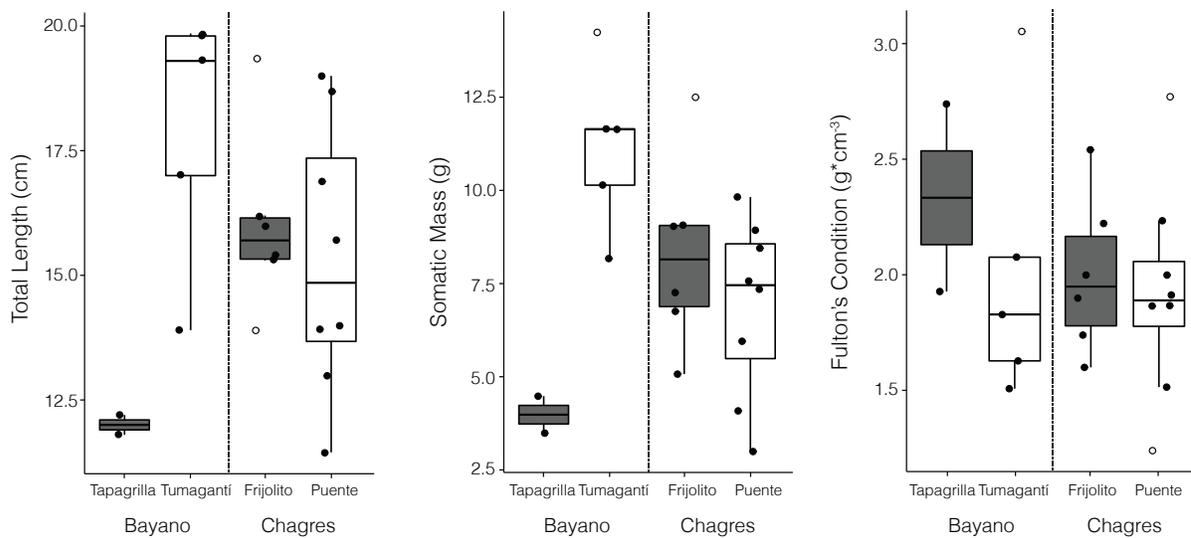


Figure 1.4. Variation in general morphological traits of *B. occidentalis* a) females ($n = 44$ fish) and b) males ($n = 21$ fish) from high and low predation risk sites within each drainage. High predation risk populations are indicated by the dark gray boxplots whilst low predation risk populations are in white. The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values respectively, while the black dots indicate individual values. The open dots represent outliers.

Ovary and oocyte analysis

Overall, the general reproductive traits under study varied significantly based on predation risk and drainage (Table 1.6). Females from low predation risk sites had ovaries that were 220% heavier and had a greater GSI than females in high predation risk sites. In Chagres, females had gonads that were 130% heavier, and a greater GSI than females from Bayano (Figure 1.5). Gonad asymmetry also varied based on predation risk, with females having larger right ovaries under low predation risk. Nevertheless, no significant interactions were observed between drainage and predation risk.

Table 1.6. Results of 2x2 Factorial Analysis of Covariance (ANCOVAs) examining the main effects of predation and drainage on gonad mass (G_w), gonadosomatic index (GSI) and gonad asymmetry (GA) of females in *B. occidentalis* in Panama.

Effect	d.f.	G_w		GSI		GA	
		F	P	F	P	F	P
Length	1	14.0	0.001*	1.38	0.25	5.30	0.03*
Predation	1	23.7	<0.0001*	35.4	<0.0001*	5.72	0.02*
Drainage	1	11.1	0.002*	13.6	0.001*	2.18	0.15
Predation x Drainage	1	1.32	0.26	1.58	0.22	2.17	0.50
Error		43		43		37	

Note: Descriptive variables are as follows: gonad mass (G_w), gonadosomatic index (GSI) and gonad asymmetry (GA). Asterisks show a significant effect of predation on observed reproductive trait ($P < 0.05$).

The absolute fecundity (AF) of the sampled *B. occidentalis* averaged at 326.97 oocytes per female (ranging from 61 to 831 oocytes), while the average relative fecundity (RF) was estimated at 56.72 ± 4.97 oocytes per gram of female body weight. I observed variation in both absolute and relative fecundity across sampling sites (Figure 1.6; Table 1.7). Significant variation in both AF ($F_{1,32} = 7.78$, $P = 0.009$) and RF ($F_{1,32} = 16.8$, $P < 0.0001$) of females was observed based on predation risk (Figure 1.5). I also observed significant variation in AF

($F_{1,32} = 9.36$, $P = 0.005$) and RF ($F_{1,32} = 9.56$, $P = 0.004$) based on drainage. However, there was no significant interaction between predation risk and drainage ($P = 0.68$ for AF, $P = 0.73$ for RF).

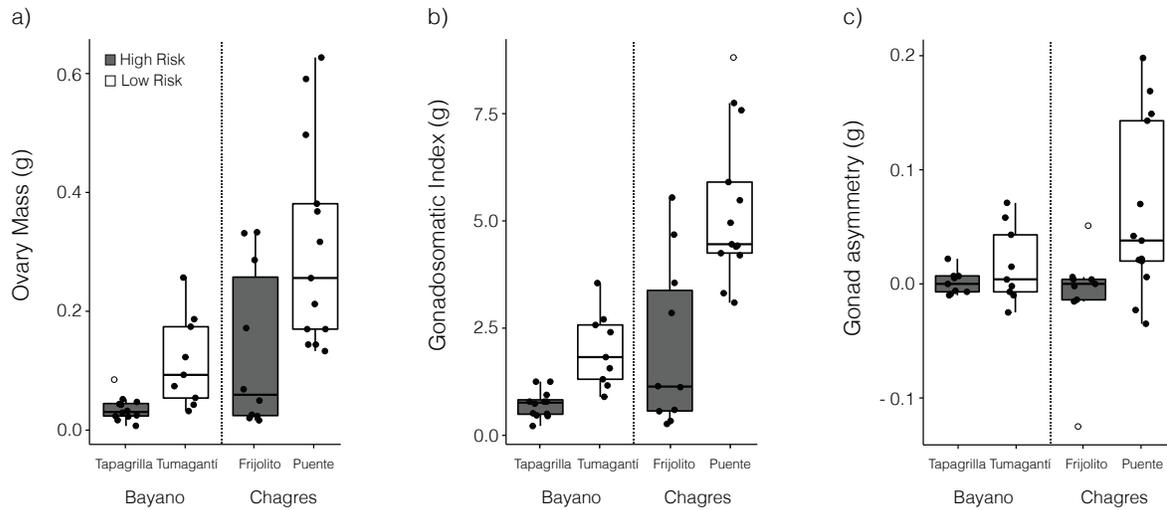


Figure 1.5. Variation of a) ovary mass ($n = 44$ fish), b) GSI ($n = 44$ fish) and c) gonad asymmetry ($n = 40$ fish) in female *B. occidentalis*. High predation risk populations are indicated by the dark gray boxplots whilst low predation risk populations are in white. The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values respectively, while the black dots indicate individual values. The open dots represent outliers.

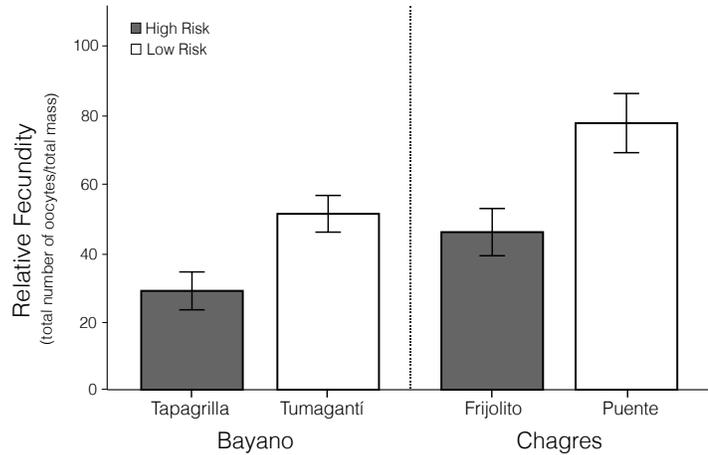


Figure 1.6. Variation in relative fecundity of *B. occidentalis* females. High predation risk females ($n = 12$ fish) are in dark gray whilst low predation risk females ($n = 21$ fish) are in white. Data are shown as Mean \pm S.E.

On average, *B. occidentalis* females had 247.8 ± 25.7 immature oocytes, 37.4 ± 49.8 maturing oocytes and 41.8 ± 37.4 mature oocytes. Oocyte quantity per maturity level varied across sampling sites (Table 1.7). I observed a significant difference in immature oocyte quantity between high predation and low predation risk populations ($F_{1,32} = 5.19$, $P = 0.030$), with females from low predation risk sites having 64.97 % more immature oocytes. However, I did not observe drainage differences ($P = 0.062$), nor did I observe a significant interaction between predation and drainage on immature oocyte quantity ($P = 0.66$). Similarly, low predation risk females had 210 % more maturing oocytes than females from high predation risk sites ($F_{1,30} = 4.23$, $P = 0.050$), with no difference based on drainage ($P = 0.10$), and no significant interaction ($P = 0.56$). Finally, I observed a significant variation in mature oocyte quantity ($F_{1,26} = 5.14$; $P = 0.033$) based on predation risk; females from low predation risk sites had 21.5% more mature oocytes. Also, I observed a significant difference in mature oocytes between the drainages ($F_{1,26} = 8.74$; $P = 0.007$), with Chagres females having 218.3% more mature oocytes than Bayano females. I also observed a significant interaction between

predation risk and drainage for mature oocytes ($F_{1,26} = 8.33$; $P = 0.008$), where further analysis revealed that in Chagres, females from low predation risk sites had less mature oocytes ($P = 0.07$); while in Bayano, females from low predation risk sites had more mature oocytes ($P = 0.51$); nevertheless, none of those differences were significant. When looking at females from high predation risk sites, Chagres females had more mature oocytes ($F_{1,8} = 32.9$; $P < 0.0001$). I did not observe a difference in number of mature oocytes between drainages ($P = 0.41$) in females from low predation risk sites.

Table 1.7. Oocyte quantity by maturity level and absolute fecundity (AF) in *B. occidentalis* females in Panamá.

	Bayano		Chagres	
	High Predation	Low Predation	High Predation	Low Predation
	Tapagrilla	Tumagantí	Frijolito	Puente
Immature (n=33)	134.6 ± 20.2 ^a	208.5 ± 40.2 ^{a, b}	204.3 ± 39.4 ^{a, b}	338.9 ± 46.4 ^b
Maturing (n=31)	4.8 ± 2.8 ^a	41.9 ± 13.3 ^{a, b}	24.0 ± 5.8 ^{a, b}	54.3 ± 19.2 ^b
Mature (n=28)	1.4 ± 0.75 ^a	28.4 ± 11.2 ^b	62.0 ± 17.0 ^b	37.4 ± 8.7 ^b
AF (n=33)	140.8 ± 21.0 ^a	278.8 ± 54.6 ^{a, b}	290.3 ± 54.7 ^{a, b}	448.0 ± 56.2 ^b

Note: Data are shown as means ± standard deviation. Letters indicate the statistical differences among sampling sites, two columns with the same letter do not differ (post-hoc comparison $P < 0.05$).

Overall, I observed variation in immature oocyte traits for *B. occidentalis* females (Figure 1.7a). Specifically, I observed a significant difference in immature oocyte diameter ($F_{2,26} = 3.83$; $P = 0.036$), immature oocyte volume ($F_{2,26} = 4.44$; $P = 0.023$) and immature oocyte surface area ($F_{2,26} = 4.33$; $P = 0.025$) across sampling sites. For all three parameters, the post-hoc test shows that females from Puente had larger immature oocytes than Tumagantí females ($P = 0.041$ for oocyte diameter; $P = 0.025$ for oocyte volume and $P = 0.028$ for oocyte surface area). I observed no significant difference in immature oocyte diameter ($P = 0.39$), volume ($P = 0.40$) or surface area ($P = 0.37$) based on predation risk. However, I did observe a

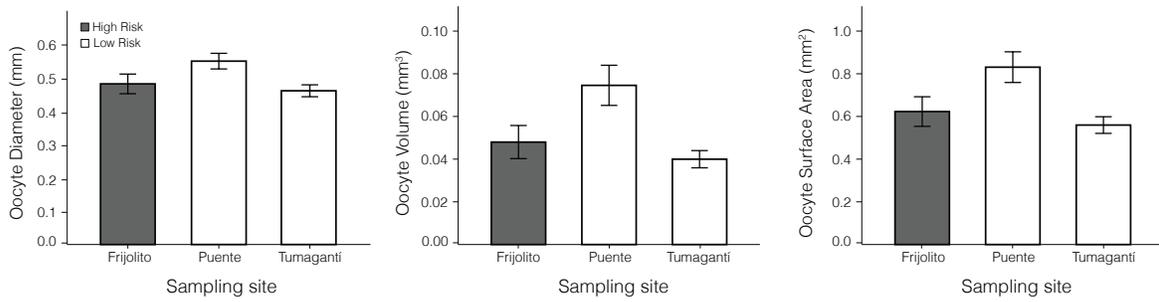
marginally significant difference in oocyte diameter based on drainage ($F_{1,26} = 4.01$; $P = 0.056$), and significant differences in oocyte volume ($F_{1,26} = 4.81$; $P = 0.038$) and oocyte surface area ($F_{1,26} = 4.60$; $P = 0.042$); with females from Chagres having larger immature oocytes than Bayano females. Within Chagres, I observed no significant difference in immature oocyte diameter ($P = 0.11$), volume ($P = 0.11$) and surface area ($P = 0.10$) between sites.

Maturing oocyte diameter ($F_{2,26} = 5.37$; $P = 0.012$), volume ($F_{2,26} = 5.10$; $P = 0.014$) and surface area ($F_{2,26} = 4.28$; $P = 0.026$) all varied significantly across sampling sites (Figure 1.7b). Post-hoc analyses show that females from Puente had greater oocyte parameters ($P = 0.014$ for diameter; $P = 0.021$ for volume; $P = 0.033$ for surface area) than females from Tumagantí. I observed no significant difference in maturing oocyte diameter ($P = 0.32$), volume ($P = 0.25$) and surface area ($P = 0.31$) based on predation risk. However, I did observe significant drainage differences in maturing oocyte diameter ($F_{1,26} = 5.28$; $P = 0.030$) and volume ($F_{1,26} = 4.37$; $P = 0.05$), where Bayano females had smaller oocyte diameters and volumes than Chagres females, but only a marginally significant difference in oocyte surface area ($P = 0.056$). Within Chagres, I observed no differences in maturing oocyte diameter ($P = 0.69$), volume ($P = 0.58$) or surface area ($P = 0.09$) between sites.

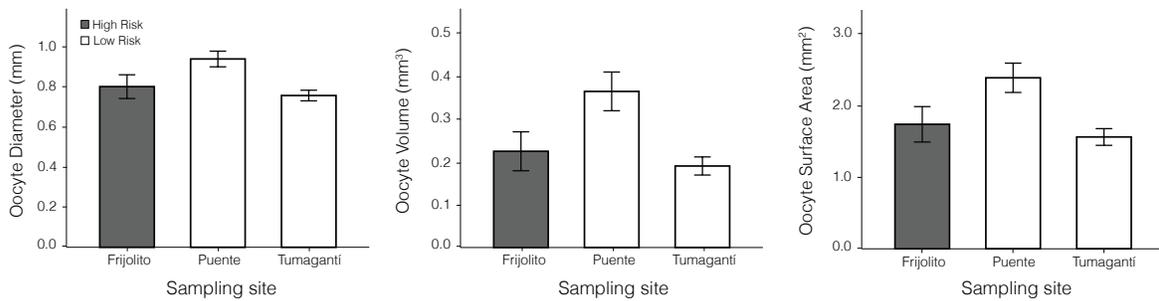
Finally, for mature oocytes (Figure 1.7c), I observed a significant difference in oocyte diameter ($F_{2,23} = 4.02$; $P = 0.033$), with females from Puente having larger oocyte diameters than females from Tumagantí ($P = 0.026$). Similarly, I observed variation in mature oocyte volume ($F_{2,23} = 3.79$; $P = 0.039$) and surface area ($F_{2,23} = 3.79$; $P = 0.039$) across sampling sites, with mature oocytes being larger in Puente females ($P = 0.031$ for both volume and surface area). I observed no variation in oocyte parameters based on predation risk (diameter: $P = 0.95$, volume: $P = 0.99$; surface area: $P = 0.99$ for surface area); however, mature oocytes in Bayano were significantly smaller in diameter ($F_{1,23} = 7.28$; $P = 0.013$), volume ($F_{1,23} = 7.05$; $P = 0.014$)

and surface area ($F_{1,23} = 7.05$; $P = 0.014$). Finally, I did not observe a variation in mature oocyte parameters based on predation risk within Chagres ($P = 0.35$ for diameter, $P = 0.40$ for volume and $P = 0.40$ for surface area).

a) Immature Oocytes



b) Maturing Oocytes



c) Mature Oocytes

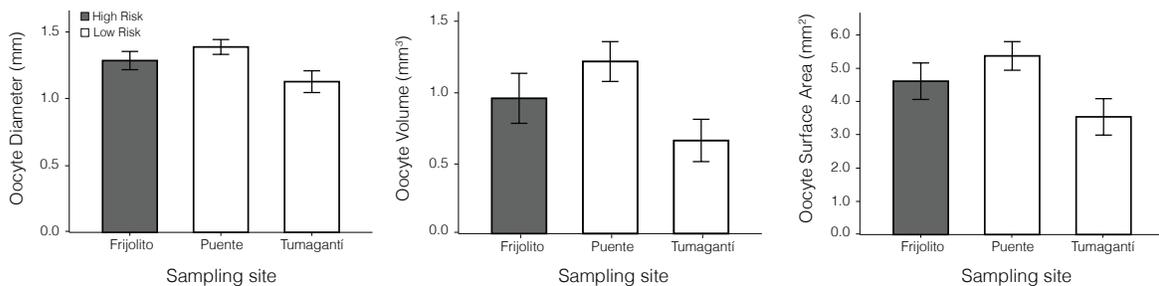


Figure 1.7. Variation in oocyte diameter, volume and surface area of *B. occidentalis* females across sampling sites. a) immature oocyte trait variation ($n = 27$ fish), b) maturing oocyte trait variation ($n = 27$ fish) and c) mature oocyte trait variation ($n = 24$ fish). High predation risk populations are represented by the dark gray bars whilst low predation risk populations are in white. Data are shown as Mean \pm S.E.

Testes and sperm analysis

Neither predation risk nor drainage had a significant effect on the general traits examined in males (Table 1.8, Figure 1.8). Interestingly, predation risk did seem to be a biologically relevant factor contributing to variation in GSI for males (Table 1.8).

Table 1.8. Results of 2x2 Factorial Analysis of Covariance (ANCOVAs) examining the main effects of predation and drainage on gonad mass (G_w), gonadosomatic index (GSI) and gonad asymmetry (GA) of males in *B. occidentalis* in Panamá.

Effect	d.f.	G_w		GSI		GA	
		F	P	F	P	F	P
Length	1	30.0	<0.0001*	12.0	0.003*	0.002	0.96
Predation	1	2.07	0.17	4.42	0.05*	0.003	0.95
Drainage	1	0.07	0.80	0.16	0.69	0.21	0.66
Predation x Drainage	1	0.43	0.52	3.17	0.10	0.50	0.49
Error		19		19		18	

Note: Descriptive variables are as follows: gonad mass (G_w), gonadosomatic index (GSI) and gonad asymmetry (GA). Asterisks show a significant effect of predation on observed reproductive trait ($P < 0.05$).

Overall, the sampled *B. occidentalis* males had sperm with heads that were on average $2.20 \pm 0.31 \mu\text{m}$ wide and $2.18 \pm 0.12 \mu\text{m}$ long, flagella that measured $21.7 \pm 3.69 \mu\text{m}$ and a total length of $23.9 \pm 3.66 \mu\text{m}$ (Appendix 3). Hydrodynamic ratio was $1.01 \pm 0.12 \mu\text{m}$ and head to tail ratio index was 11.04% on average.

Sperm morphometry analyses revealed that there were no significant differences in head width ($P = 0.36$), head length ($P = 0.56$) nor flagellum length ($P = 0.31$), sperm total length ($P = 0.29$), hydrodynamic ratio ($P = 0.49$) and head length to flagellum length ($P = 0.54$) amongst sampling sites (Figure 1.9). Predation risk also did not have a significant effect on any of the observed traits ($P = 0.14$ HW; $P = 0.34$ HL; $P = 0.496$ FL; $P = 0.45$ SL; $P = 0.24$ HR; $P = 0.44$ HL: FL).

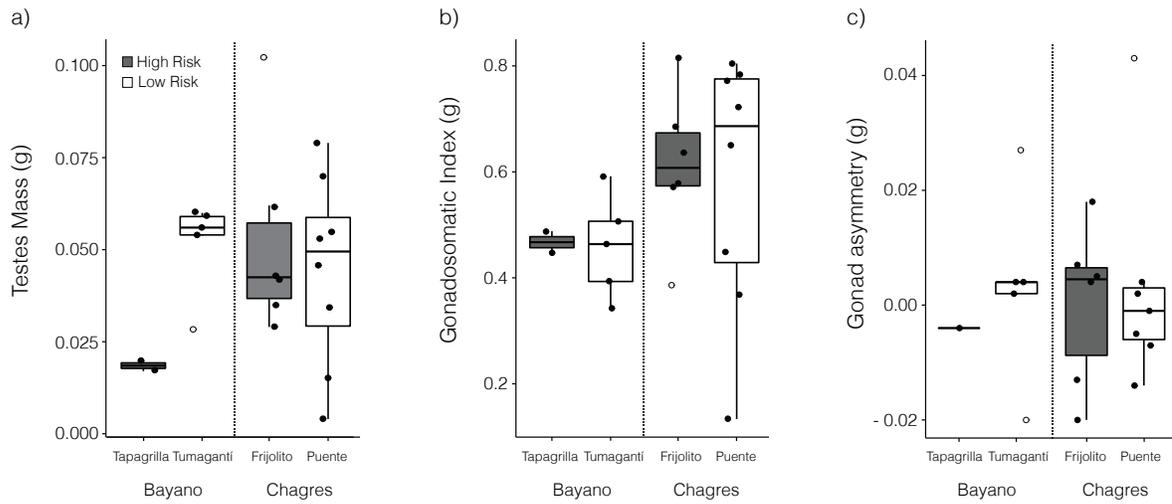


Figure 1.8. Comparison of a) testes mass ($n = 21$ fish), b) GSI ($n = 21$ fish) and c) gonad asymmetry ($n = 19$ fish) in male *B. occidentalis*. The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values respectively, while the black dots indicate individual values. The open dots represent outliers.

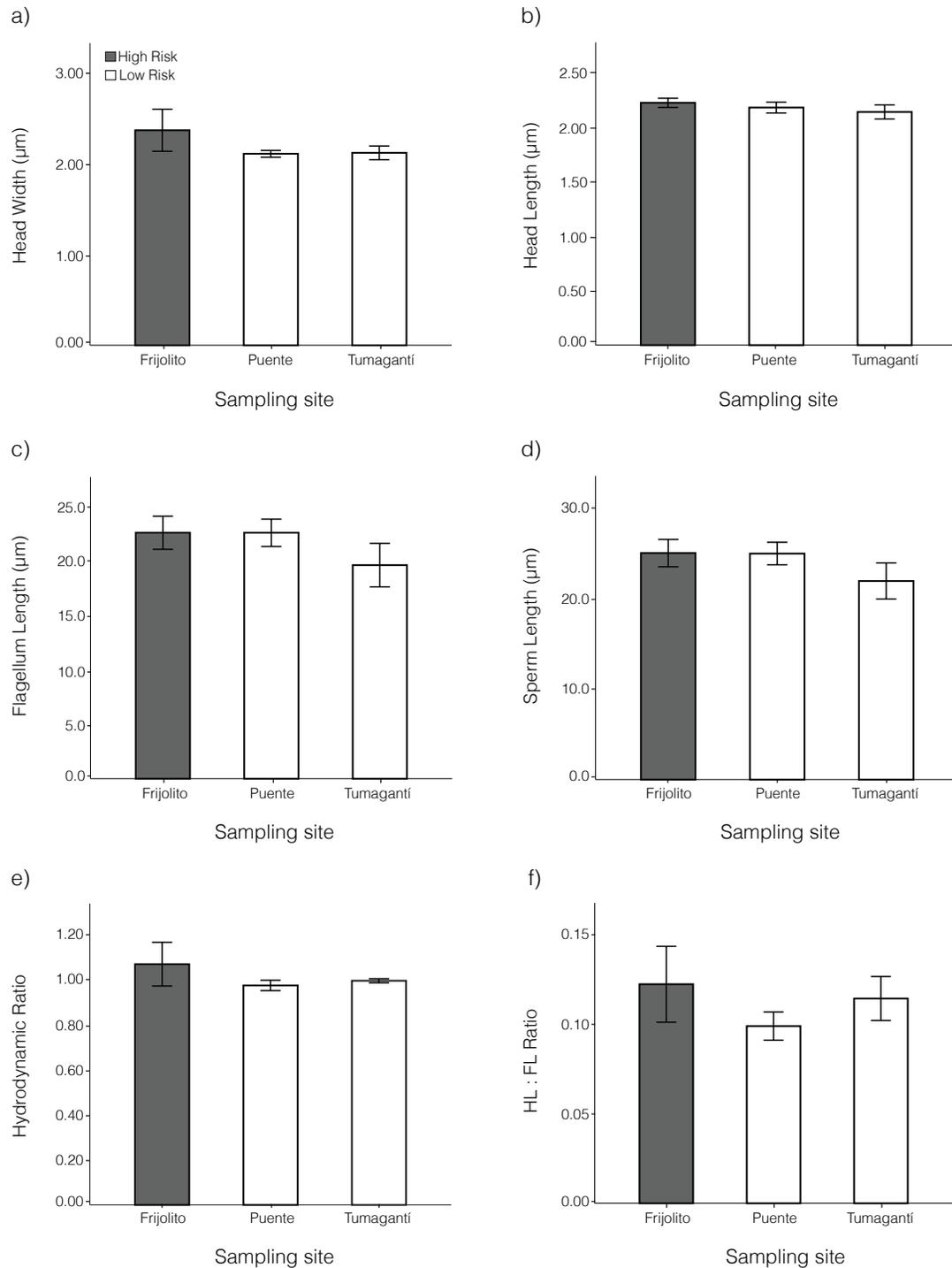


Figure 1.9. Variation in sperm morphometry traits of *B. occidentalis* across sampling sites ($n = 16$ fish). a) Head width, b) head length, c) flagellum length, d) sperm length, e) hydrodynamic ratio f) head to flagellum length ratio (HL: FL). High predation risk fish are indicated by the dark gray bars whilst low predation risk fish are shown in white. Data are shown as Mean \pm S.E.

DISCUSSION

Overall, my results support previous studies showing sexual dimorphism in *B. occidentalis* (Hagedorn and Carr, 1985; Hagedorn, 1988), with males being significantly longer and heavier than females. Because the electric organ is situated along the body axis of the knifefish (Appendix 4), all the way through to the tip of the tail (Hopkins, 1999; Stoddard *et al.*, 1999), the electric organ discharges (EODs) generated during the reproductive season reflect the anatomical differences in tail morphology. The larger tails observed in male *B. occidentalis* are a result of a greater proportion of electrocytes present in the electric organ (Hopkins, 1999; Giora *et al.*, 2014), and the signals generated have been associated with increased attractiveness to females (Curtis and Stoddard, 2003; Machnik and Kramer, 2008; Crampton, 2019) and mating success (Hagedorn, 1988; Giora *et al.*, 2014). Hence, studies have demonstrated that EOD generation is more expensive for males than females (Salazar and Stoddard, 2008; Stoddard and Salazar, 2011), with male knifefish budgeting up to 22% of daily oxygen intake on signal production alone (Salazar *et al.*, 2013). The high energy budgeted by males into electric signalling suggests that EOD production may result in trade-offs with other expensive processes, such as somatic growth (Stoddard and Salazar, 2011), particularly under the stress of predation risk.

In my study specifically, male total length and mass varied under increased predation risk, with males from high predation risk sites being shorter and lighter. Although the observed variation could have been due to a small sample size of low predation risk populations, these results also suggest that predation risk can affect male investment to somatic growth. Non-lethal interactions between prey and predators can lead to prey behaviour change (Peckarsky and McIntosh, 1998), such as signal cloaking observed in electric fishes. For instance, *B.*

occidentalis have been shown to modify their EODs in order to remain outside of the detection range of their electrosensing predator, *R. quelen* (Stoddard and Markham, 2008; Tran, 2014). This behavioural change can result in trade-offs between predator avoidance mechanisms and mating, habitat selection and foraging (Lima and Dill, 1990), the latter ultimately resulting in reduced body size. My study also reveals that predation had an effect on Fulton's condition factor in female *B. occidentalis*. Fulton's condition is a popular condition index used in fisheries management and fish ecology (Stevenson and Woods, 2006). Because *B. occidentalis* have an elongated body shape compared to the standard salmonid fish shape (Jones *et al.*, 1999), females with lower K values are longer in proportion to their body weight. Females under high predation risk were overall shorter for their mass than females from low predation risk sites, suggesting that females may also be experiencing trade-offs under predation risk. Although predator-induced changes in growth rates and body morphology have been reported in many fish species (Fraser and Gilliam, 1992; Arendt and Reznick, 2005; Sharpe *et al.*, 2012; Ingley *et al.*, 2014), future studies should explore whether this is associated to reduced foraging, or trade-offs between signal cloaking in electric fishes under varying degrees of predation risk.

Female reproductive traits

Reproduction and somatic maintenance require important and often conflicting energy investments (Cody, 1966; Jensen, 1996; Brosset *et al.*, 2016; Baulier *et al.*, 2017; Johansson *et al.*, 2018). For females in particular, reproduction and gonadal investments take up a great proportion of the overall energetic budget (Reznick, 1992; Jørgensen *et al.*, 2008; Baulier *et al.*, 2017; Pauly, 2019). Some additional factors, such as predation risk, can also limit the amount of energy invested into these expensive processes, resulting in trade-offs between reproductive investments, somatic growth and survival (Stearns, 1989; Brosset *et al.*, 2016; Chamberlain *et*

al., 2017; Possenti *et al.*, 2019). Predation risk is an important selective force that has been shown to drive the evolution of multiple physiological and morphological traits (Dunlap *et al.*, 2016; Walsh *et al.*, 2016; Taylor and Dunn, 2017). In terms of reproduction, increased predation has been linked to changes in age at maturity (Reznick *et al.*, 2001; Sharpe *et al.*, 2012), fecundity (Billman *et al.*, 2011; Grégoir *et al.*, 2018) and clutch and egg size (Doligez and Clobert, 2003; Mukherjee *et al.*, 2014; Possenti *et al.*, 2019), among others. My study reveals a clear association between predation risk environment and reproductive investment across female *B. occidentalis* populations.

Life-history theory predicts that organisms allocate resources to reproduction over time, in response to the costs and fitness benefits of current reproduction versus future reproductive opportunities (Brommer, 2000; Harris and Uller, 2009; Belk and Tuckfield, 2010). Consequently, females facing higher predation risk, thus lower probability of future reproduction, are expected to increase their reproductive effort and investments into offspring, at the expense of oocyte quality according to the “terminal investment hypothesis” (Schreck, 2010; Fisher and Blomberg, 2011; Sharpe *et al.*, 2012). The gonadosomatic index (GSI) is a widely used metric representing the proportion of body tissue devoted to gamete production, providing insight on a female’s reproductive capacity and gonadal development (Yoneda *et al.*, 2013; Flores *et al.*, 2015; Parker *et al.*, 2018). Stressed females should therefore display higher GSIs, reflecting the greater investment into gonad size as a result of increased reproductive effort. Contrary to these predictions, *B. occidentalis* females under high predation risk had lower GSIs and lighter ovaries in both drainages, suggesting a reduction in reproductive effort in those sites. Similar results have been reported in other fish species (Fraser and Gilliam, 1992; Mukherjee *et al.*, 2014; Heins *et al.*, 2016) and in other animals (Creel *et al.*, 2007; Travers *et*

al., 2010), suggesting that reproduction is a highly plastic process, and that predation risk can lead organisms to respond with a variety of life history strategies to increase fitness.

Fecundity, another important measure of an individual's reproductive capacity (Oliveira *et al.*, 2010), has also been shown to vary based on predation risk in multiple fish species (Reznick and Endler, 1982; Reznick *et al.*, 2001; Grégoir *et al.*, 2018). For instance, southern leatherside chubs (*Lepidomeda aliciae*) from high quality habitats produce fewer oocytes (absolute fecundity) when introduced to predators (Billman *et al.*, 2011). In my study, females from high predation risk sites also had lower fecundities (absolute and relative), with lower oocyte quantities per maturity stage, again demonstrating that increased predation risk is inversely linked to reproductive output in this species.

The effects of predation risk on oocyte size have seldom been studied and the available research provides conflicting results (Possenti *et al.*, 2019). Despite several studies linking oocyte size positively with traits associated with increased offspring survival (Einum *et al.*, 2002; Segers and Taborsky, 2012; Hedeholm *et al.*, 2017), I observed no difference in oocyte volume, diameter or surface area between predation risk sites. Similar results have been reported in other tropical fish species (Sharpe *et al.*, 2012; Heins *et al.*, 2016). My results suggest that despite the potential physiological stress experienced by *B. occidentalis* females under increased predator interactions, these effects do not seem to cause variations in oocyte size, but rather oocyte quantity. Typically, female fish can either produce multiple small oocytes, or fewer large oocytes (Heinimaa and Heinimaa, 2004; Quinn *et al.*, 2011; Lasne *et al.*, 2018). Under varying conditions, females trade-off oocyte quality (size) with oocyte quantity and offspring survival (Einum and Fleming, 2000; Einum *et al.*, 2002). These trade-offs allow females to adjust their reproductive potential (Heinimaa and Heinimaa, 2004). Larger oocytes have been linked to larger juveniles, and increased offspring survival (Einum

and Fleming, 2000; Leblanc *et al.*, 2014; Smalås *et al.*, 2017), while higher fecundity leads to more copies of parental genes in future generations, favoring parental fitness (Lasne *et al.*, 2018). In low predation risk sites, it is possible that females are prioritizing oocyte number, thereby maximizing maternal fitness.

Predation risk has been shown to affect reproduction both directly (physiological stress) and indirectly (risk effects). Physiological stressors can contribute to changes in metabolism and immune response due to increased hormone production in prey species (Clinchy *et al.*, 2013). Because physiological stress is an adaptive response geared to increase survival during life-threatening situations, stress response generally involves the allocation of resources to those processes, while interfering with essential body functions (Hawlena and Schmitz, 2010). On the other hand, indirect effects generally refer to behavioural changes resulting from predation threat that consequently affect prey biology (Hawlena and Schmitz, 2010; Mukherjee *et al.*, 2014). For example, foraging rate and habitat choice have been shown to vary under predation risk (Magnhagen, 1991; Walsh *et al.*, 2016). Fish require rich protein and lipid content for reproduction (Woodhead, 1960; Volkoff and London, 2018); however, when faced with predation, prey might prioritize safety by reducing foraging rates or modifying habitat use for protection or hiding (Eggers *et al.*, 2006; Mukherjee *et al.*, 2014). Reduced nutrient consumption can lead to insufficient energy accumulation, limiting allocation towards reproduction, growth and other life history traits that are not directly tied to organism survival (Mukherjee *et al.*, 2014; Hedeholm *et al.*, 2017). Various studies have shown that fish undergoing gonadal maturation during spawning periods of lower food availability use their somatic energy reserves, leading to trade-offs with overall growth (Nandikeswari, 2016). The reduced ovary size, fecundity and GSI observed in females could be linked to reduced foraging with the onset of increased predation risk, although future studies should confirm this hypothesis

by investigating resource availability, as well as stomach contents in natural populations, or by controlling feeding habits in laboratory studies.

In my study, females under low predation risk had a higher prevalence of gonad asymmetry, suggesting that fluctuations are not a result of predation stress in this species of knifefish. There is increasing evidence of natural occurrences of fluctuating asymmetry in certain fish populations (Allenbach, 2011), such as in multiple female stingrays species (Wourms, 1977; Da Silva *et al.*, 2017). It is hypothesized that having two large functional gonads is costly, and that smaller gonads only get larger to compensate for the lack of function in the typically larger gonad (Moller, 1994; Da Silva *et al.*, 2017). One can suggest that individuals might be prioritizing one well developed and functional gonad rather than two less formed gonads. Contrary to my predictions, females from Puente and Tumagantí low predation sites often presented atrophied left ovaries. There are reports of gonad degeneration in mature fish (McMillan, 2010; Miyashita and Palmer, 2014). The occurrence of ovarian atrophy in mature fishes has often been associated with external perturbations, such as increased levels of endocrine disruptive compounds (Arcand-Hoy and Benson, 1998; Senarat *et al.*, 2017), lead nitrate (Adeyemo, 2008), parasitic infections (Kaur *et al.*, 2013) and temperature variation (Luksiene and Sandström, 1994).

Another plausible explanation to the opposite results observed is that females may be “downregulating” fecundity (Tyler and Sumpter, 1996; Rideout and Tomkiewicz, 2011). It has been suggested that at the beginning of the reproductive cycle, females may induce a greater number of oocytes into vitellogenesis than necessary (Kjesbu, 2009). If energy is not sufficient, oocyte atresia, the reabsorption of vitellogenic oocytes, can occur (Skjæraasen *et al.*, 2009), allowing females to adjust the number of oocytes to the amount of available stored energy (Rideout and Tomkiewicz, 2011). This phenomenon has been observed in other teleosts

(Saidapur, 1978; Kjesbu, 2009; Skjæraasen *et al.*, 2009), and evidence suggests it can occur at any stage of development (Tyler *et al.*, 1990). Although my results do not indicate that the observed asymmetry is induced by predation stress, this does not mean that females at the low predation risk sites are not subjected to other stressors that may cause gonad variation. Future studies should determine the presence of potential stressors, such as pollution, habitat quality, level of dissolved oxygen and temperature, all of which play important roles in gonad asymmetry (Groenendijk, 1998; Sopinka *et al.*, 2012; Benderlioglu and Dow, 2017). Although I only observed atresia in populations with high fecundity (and low predation risk), this seems a very interesting aspect to further explore in this species, and other Gymnotiformes.

It is also important to note that I was unable to perform oocyte and ovary analyses with *B. occidentalis* sampled from the high predation risk site Tapagrilla. Although females were generally lighter and shorter at this site, the prevalence of juveniles sampled was much higher. Interestingly, individuals that would have reached oocyte maturity at other sites based on body mass and length, did not display gonad maturity or had immature ovaries (only immature oocytes present) in Tapagrilla. Because of this, I was not able to compare specific reproductive parameters. Nevertheless, it is valuable that I mention this observation, as it may indicate that either predation risk, or other external factors specific to that site contribute to slower reproductive maturity in this species. Future studies should also explore whether this trend is observed annually and determine what factors may be contributing to this observed variation.

Male reproductive investment traits

Male reproductive success is heavily dependent on the production of high-quality sperm (Van Look and Kime, 2003; Casselman *et al.*, 2006; Beirão *et al.*, 2019; Mishu *et al.*, 2020). As a result, substantial effort has been devoted to explaining sperm morphological diversity across a

variety of species, including fishes. To date, few studies have looked into characterizing gymnotiform male reproductive biology (França *et al.*, 2007; França *et al.*, 2009; Giora and Burns, 2011; Vergílio *et al.*, 2013), and data on sperm morphology and overall reproductive biology of *B. occidentalis* are lacking. To my knowledge, my study is the first to report the reproductive investment traits of male gymnotiforms under predation risk.

Sexual selection is an important force shaping male reproductive traits (Miller and Svensson, 2014; Devigili *et al.*, 2019). A fundamental assumption of sexual selection theory is that traits associated with male competitive fertilization success, such as testes size and sperm production, should be favored (Parker, 1970; Andersson, 1994; Burness *et al.*, 2008). It is hypothesized that testes size is a direct reflection of male investment to sperm production (Moller, 1989; Schärer and Vizoso, 2007; Immler *et al.*, 2011; Simmons *et al.*, 2017), and there is mounting evidence linking larger testes to higher sperm quality and quantity (Burness *et al.*, 2008; Lüpold *et al.*, 2020), thus higher reproductive success and fitness. In my study, gonad mass represented 0.5% of male body mass, and I observed no difference in testes mass between the predation risk populations, despite males from low predation risk sites being longer and heavier. My results also show no significant difference in testes asymmetry, a trait linked to increased environmental stressors in fishes (Sopinka *et al.*, 2012), suggesting that sampled males seemingly invest the same amount into both gonads regardless of potential external stress.

However, my results do show a biologically relevant difference in male GSI, with males from low predation risk sites having a slightly higher GSI. There are often conflicting costs between reproduction and predator avoidance (Magnhagen, 1991). As previously discussed, there is a high associated cost linked to EOD generation in male knifefishes (Salazar *et al.*, 2013; Crampton, 2019), and electric signalling is tied to caudal filament length and mate attraction (Hopkins, 1999). However, masculinized EODs make males vulnerable to predators

(Stoddard and Markham, 2008; Tran, 2014), and high predation risk individuals have been shown to modify their electric signal to evade detection by predators (Tran, 2014). This adaptive mechanism can in turn reduce attractiveness to females, reducing mating opportunities (Stoddard and Markham, 2008), while also reducing the energy available for reproduction (Grégoir *et al.*, 2018). It is possible that male *B. occidentalis* trade off growth, EOD production and mate attraction for GSI under predation stress, although more research needs to be done to confirm this hypothesis and elucidate the mechanisms by which this variation operates.

The presence of intraspecific competition has also been shown to have an effect on testes development and sperm swimming parameters (Schreck *et al.*, 2001; Burness *et al.*, 2008; Hayward and Gillooly, 2011; Martinez *et al.*, 2015). Fish that undergo competition due to high population density or mating system type tend to invest more into spermatogenesis to increase their fitness, resulting in higher gonadosomatic index (GSI) than males from less competitive systems (Stockley *et al.*, 1997; Awata *et al.*, 2008). Because I observed a marginal difference in GSI between predation risk populations, I suggest that males in low predation risk sites might be in more competitive systems than their high predation risk counterparts.

I also investigated variation in specific sperm morphological parameters that have been associated with male reproductive fitness. There is great diversity in sperm morphology traits within and between species (Stockley *et al.*, 1997; Gasparini *et al.*, 2010; Martinez *et al.*, 2015). In fishes, sperm length can range from 13 μm , as observed in the flathead grey mullet (*Mugil cephalus*), to over 100 μm long in the channel catfish, *Ictalurus punctatus* (Thünken *et al.*, 2007). In my study, spermatozoa were characterized by a spherical head, visible round nucleus, midpiece and single flagellum, as observed in related species *B. pinnicaudatus*, *B. draco*, *B. gauderio* and *B. bombilla* (França *et al.*, 2007; Giora and Burns, 2011). My results showed that the sampled *B. occidentalis* males had spermatozoon that had an average length of 24 μm long,

reflecting results reported in another gymnotiform species, *Gymnotus carapo*, where sperm had a total length of 26 μm and the sperm head has a diameter of 2.3 μm (Vergílio *et al.*, 2013). One of the most studied sperm traits in relation to fertilization success is flagellum length, as observed in the rainbowfish *Melanotaenia australis* (Simpson *et al.*, 2014). Flagellum length generates the thrust force necessary to propel sperm (Katz *et al.*, 1989), and is thus closely associated to fertilization success (Fitzpatrick *et al.*, 2009; Fitzpatrick *et al.*, 2010). My results, however, indicate that sperm flagellum length did not differ between sampling sites, nor based on predation risk within the studied *B. occidentalis* populations, suggesting that factors other than predation risk could be more important drivers for this sperm trait selection.

Although the contribution of flagellum length is well documented, it is unlikely that ejaculate components evolve independently, and thus should not be examined in isolation (Gómez Montoto *et al.*, 2011; Immler *et al.*, 2011; Lüpold, 2013; Liao *et al.*, 2018). Like flagellum length, sperm head morphology has been linked with male reproductive fitness (Simpson *et al.*, 2014). Hydrodynamic ratio is a widely used sperm performance parameter (Humphries *et al.*, 2008b), and has been shown to be an important contributor to sperm overall swimming trajectory, as seen in guppies (Pitcher *et al.*, 2007; Gadêlha *et al.*, 2010; Simpson *et al.*, 2014). Also, smaller sperm heads have been associated with decreased overall fitness (Sopinka *et al.*, 2012), particularly if smaller sperm heads are a result of DNA damage, as observed in the rainbow trout, *Oncorhynchus mykiss* (Labbe *et al.*, 2001). Contrary to my hypothesis, none of the sperm morphology traits varied across sampling sites or between predation risks, despite an increasing body of work suggesting that sperm traits can vary according to environmental variables (Devigili *et al.*, 2019; Rahman *et al.*, 2020) and sperm competition (Schreck *et al.*, 2001; Burness *et al.*, 2008; Hayward and Gillooly, 2011; Martinez *et al.*, 2015). Sperm competition is a powerful evolutionary force that selects for male

reproductive traits, particularly sperm morphology, that ultimately lead to competitive fertilization success (Parker, 1970; Parker and Pizzari, 2010; Simmons and Fitzpatrick, 2012; Simpson *et al.*, 2014). Knifefish are external fertilizers (Vergilio *et al.*, 2013); however, it has been suggested that one-on-one interactions occur during courtship (Curtis and Stoddard, 2003). This mating system would be less competitive, potentially explaining the lack of significant variation in sperm traits across sampling sites. The absence of variation could also be due to the low sample size (the highest power observed was for head width at 0.205), especially when omitting outliers and Tapagrilla, where I was unable to extract milt from the testes. Even though studies with similar sample sizes have shown that variation exists in sperm traits sizes (Elgee *et al.*, 2010; Perrault, 2015), perhaps my samples sizes were not large enough to observe variation in this particular species. When calculating the coefficient of variability of sperm traits per individual, I observed a low-level dispersion around the means for the head traits (< 25 %), but a moderately high level of variability with the flagellum traits (19 % to 52 %). It is therefore possible that high intra-individual variability in sperm morphological traits impeded me to detect a difference when comparing among individuals and sites. Finally, my study system included two replicates for both drainages and predation risks, which could have been a potential limitation as typically, three replicates are targeted for statistical significance, and biological relevance (Jobling, 1995; Vaux *et al.*, 2012).

Conclusion

Research on the reproductive biology of fishes has sought to advance our knowledge on the different qualities and traits driving reproductive investment patterns. To my knowledge, my study was the first to report reproductive biology in natural populations of *B. occidentalis* under predation risk. Overall, my study reveals a clear association between predation risk environment, geographical isolation (i.e., drainage) and female reproductive strategies. The lower reproductive output observed in high predation risk populations could be a result of a variety of plastic or adaptive responses in females. Shifts in behaviour (reduced foraging or increased hiding) or increased physiological stress can result in heightened survival during life-threatening situations, leading to trade-offs with reproduction and growth. Future studies should consider resource availability, nutrient quality and quantity, and stomach contents in natural populations, to determine whether the observed reduction in reproductive output is a result of reduced nutrient intake rather than actual direct predation stress.

My study is also the first to report the reproductive biology of male *B. occidentalis*. Spermatozoa were characterized by a spherical head, visible round nucleus, midpiece and single flagellum. I observed no difference in testes traits or sperm morphological traits based on site, predation risk or geographic isolation. Future studies should consider exploring other environmental factors such as habitat complexity, food quantity and quality, as well as water temperature to see whether and how external factors contribute to variation in male reproductive traits. Finally, by studying natural populations of *B. occidentalis* under a variety of socially complex environments, we could determine whether other external factors contribute to sperm trait variation by way of increased sperm competition.

CHAPTER II: Brain mass variation and allometry in *Brachyhypopomus occidentalis*

ABSTRACT

Characterizing the factors that shape variation in brain size in natural populations is crucial to understand the evolution of brain size in animals. I explored how extrinsic factors such as predation risk and drainage contribute to brain mass variation in the electric fish *Brachyhypopomus occidentalis*. I also explored how ontogenetic scaling relationships influence brain size, and how this association may be affected by predation. Fish were sampled from a high and low predation risk site within two independent river drainages in eastern and central Panama. I found significant differences in brain mass associated with predation risk, drainage and sex, with fish from low predation risk sites showing significantly heavier brains. Fish from Chagres had significantly heavier brains than fish from Bayano, and fish from the high predation site within Bayano had the smallest brains. Sex category also had a significant effect on absolute brain mass, with the trends differing in drainages. In Chagres, males and females had significantly heavier brains than juveniles, while in Bayano, males had heavier brains than both females and juveniles. Brain-body size allometric regressions followed the same trends, with fish from low predation risk sites having steeper ontogenetic slopes than fish under high predation risk, and Chagres populations having steeper ontogenetic allometric slopes than Bayano populations. Overall, my results suggest that predation risk is an important driver of brain mass variation in electric fishes, however, its effect can vary with sex, developmental stage and geographical isolation. I further suggest that this variation in brain mass may be attributed to multiple extrinsic and intrinsic factors, including differences in environmental complexity, social interaction and individual internal constraints. This study highlights predation risk as a key selective pressure in brain size evolution.

INTRODUCTION

Variation in brain size plays a crucial role in organismal performance, with larger brains often associated with more advanced cognitive abilities (Safi *et al.*, 2005; Gonda *et al.*, 2013; Isler, 2013; Buechel *et al.*, 2018; Fong *et al.*, 2019). Yet, despite remarkable variation in brain anatomy and size in animals (Jerison, 1973; Walsh *et al.*, 2016; Moore and DeVoogd, 2017), characterizing the selective forces that shape the evolution of brain size remains an open question (Bauchot *et al.*, 1977; Gonzalez-Voyer *et al.*, 2009; Isler and van Schaik, 2009; Liao *et al.*, 2015; van der Bijl *et al.*, 2015; Luo *et al.*, 2017; Dunlap *et al.*, 2019). In particular, how intrinsic factors interact with the external environment to determine variation in brain size is difficult to disentangle (Cheverud, 1982; Eifert *et al.*, 2015; Kotrschal *et al.*, 2019), and this is particularly challenging in the context of wild populations (Gonda *et al.*, 2011; 2013; Eifert *et al.*, 2015). Here, I consider this interaction by assessing brain mass variation and brain-body allometry in the context of predation in wild populations of the electric fish *Brachyhypopomus occidentalis* in Panama.

When considering intrinsic factors, variation in brain size is closely associated with body size within and across a variety of species (Jerison, 1973; Bauchot *et al.*, 1977; Cheverud, 1982; Gonda *et al.*, 2011). In vertebrates, brain size scales allometrically with body size, and this association can be expressed as a power law (Lande, 1979; Salas *et al.*, 2015):

$$\text{brain size} = a (\text{body size})^b$$

where a and b are constants, or logarithmically:

$$\log(\text{brain size}) = b * \log(\text{body size}) + \log(a)$$

where b is the allometric slope, and a is the intercept. It is widely thought that this allometric relationship is maintained by strict developmental mechanisms, resulting in evolutionary constraints on independent brain size variation (Smith *et al.*, 1985; Tsuboi *et al.*, 2016).

When considering the external environment, a variety of factors are likely to drive the evolution of brain size. For instance, brain size variation has been directly linked to changes in food availability and quality (Striedter, 2005; Kotrschal *et al.*, 2015b), habitat structure (Safi and Dechmann, 2005; Liao *et al.*, 2015), social environment (Gonda *et al.*, 2009; Gonzalez-Voyer *et al.*, 2009), environmental complexity (Pollen *et al.*, 2007; Salvanes *et al.*, 2013; Fong *et al.*, 2019) and even captive rearing (Gonda *et al.*, 2011; Dunlap *et al.*, 2017). Of particular interest for my research is the effect of predation risk on brain size variation (Gonda *et al.*, 2012; Kotrschal *et al.*, 2015a; Samuk *et al.*, 2018; Dunlap *et al.*, 2019; Mitchell *et al.*, 2020). For example, predation pressure has been associated with a reduction in olfactory bulb size in nine-spine sticklebacks, *Pungitius pungitius* (Gonda *et al.*, 2012), and a reduction of the telencephalon and optic bulb in the three-spine sticklebacks, *Gasterosteus aculeatus* (Samuk *et al.*, 2018). In the Trinidadian killifish (*Rivulus hartii*), increased predation risk selected for males with significantly smaller brains (Walsh *et al.*, 2016). In contrast, female guppies with larger brains showed increased survival under perceived threat (Kotrschal *et al.*, 2015a), suggesting that predation risk is a complex, yet important driver for brain size variation.

Electric fish are good model organisms to study the factors driving brain size evolution. Similarly to other fish, they experience continuous growth throughout all life stages, and this pattern extends to the nervous system (Leyhausen *et al.*, 1987), where lifelong neurogenesis occurs in the brain, spinal cord and other sensory organs (Zupanc, 2006; Harahush *et al.*, 2009; Lisney *et al.*, 2017). A consequence of continuous neurogenesis is a highly plastic brain, and thus a quick responsiveness to a variety of environmental conditions (Striedter, 2005; Kotrschal

et al., 2013b; Tsuboi *et al.*, 2014; van der Bijl *et al.*, 2015; Lisney *et al.*, 2017). For my study, I used the electric fish *Brachyhypopomus occidentalis* (Regan, 1914). Like other gymnotiforms, *B. occidentalis* produces electric organ discharges (EODs) for both navigation and communication (Hagedorn, 1988; Stoddard *et al.*, 1999; Moller, 2002; Crampton, 2019). EOD generation has an associated energetic cost of up to 30% of their Standard Metabolic Rate, SMR (Salazar *et al.*, 2013; Reddon *et al.*, 2018), which may induce trade-offs among brain size, predation risk and electrocommunication. In fact, previous studies in natural populations of *B. occidentalis* found a negative correlation between the forebrain cell proliferation and high predation risk (Dunlap *et al.*, 2016). Similarly, in the related species *B. gauderio*, laboratory-induced predation risk resulted in a decrease in cell proliferation; both in the telencephalon and diencephalon (Dunlap *et al.*, 2017). While these previous studies have looked at the rate at which specific brain regions generate new cells in response to predation risk, few studies on knifefish have looked at variation in overall brain size under natural conditions, and how this variation may be influenced by both predation risk and allometric scaling.

Thus, the aim of my study was to explore whether predation risk contributes to overall brain mass variation in four natural populations of *B. occidentalis* from two different drainages in Panamá. I further assess how ontogenetic scaling relationships influence brain size, and how this association may vary in the context of predation. Because brain size variation has been linked to biotic and abiotic factors (see above), I hypothesized that predation risk induces variation in overall brain mass in natural populations of *B. occidentalis*. Based on this hypothesis, I made the following predictions:

- 1) Brain mass would differ among sampling sites and river drainages.
- 2) *B. occidentalis* from high predation risk sites would display smaller brains.

- 3) If differences in brain mass are observed among drainages and sites, this same variation will be observed with the brain-body ontogenetic allometry.
- 4) I should observe variation in brain-body scaling relationships between populations under differing predation risks.

MATERIALS AND METHODS

The fish captured and processed in Chapter I are the same used in this chapter. Please refer to “Sampling sites and fish capture” and “Fish processing” for more information.

Brain processing

Brains were carefully dissected from the skull of each fish. Special attention was paid to keep the integrity of the different sections of the brain, including the optic nerve. I measured wet brain mass (± 0.0001 g) using a SmartWeigh® High Precision Milligram Jewelry Scale GEM50. Finally, brains were placed in cryotubes and preserved in buffered formalin 10%.

STATISTICAL ANALYSES

I analyzed all of the data using IBM SPSS Statistics 24. All data were natural log transformed to meet the assumptions for normality and heterogeneity of variance. To assess variation in morphological traits (fish total length, fish total mass, Fulton’s index, and Ratio index) across sampling sites, I performed a one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc tests. I also performed ANOVAs to test the main effect of predation risk on each morphological trait. For brain mass analyses, I performed a 2x2x3 (predation x drainage x sex category) factorial ANOVA, followed by one-way ANOVAs to determine the main effects of predation and sex category within each independent drainage. To determine the best model

predicting variation in fish brain mass, I performed a stepwise regression analysis that included the four morphological traits. Brain-body mass scaling relationships in logarithmic coordinates were plotted for all individuals to determine the pattern of ontogenetic allometry. Ontogenetic allometric regressions were conducted across populations, by predation risk, by drainage and by sex category. The statistical comparison of the allometric slopes was done following the method described by Wuensch (2019). Differences were considered significantly different when $P < 0.05$.

RESULTS

Morphological variation across sampling sites

I found significant morphological variation across sampling sites (Table 2.1). Specifically, I observed significant differences among sites in total length ($F_{3,82} = 8.32$; $P < 0.0001$), total body mass ($F_{3,82} = 5.28$; $P = 0.002$) and somatic mass ($F_{3,82} = 2.85$; $P = 0.043$). I also observed significant variation in Fulton's condition ($F_{3,82} = 4.26$; $P = 0.008$) as well as body Ratio Index ($F_{3,82} = 3.33$; $P = 0.027$).

Table 2.1. Morphological traits of *Brachyhypopomus occidentalis* from four sampling sites in Panamá.

	Bayano		Chagres	
	High Predation	Low Predation	High Predation	Low Predation
	Tapagrilla (n = 22)	Tumagantí (n = 14)	Frijolito (n = 25)	Puente (n = 21)
T_L (cm)	12.3 ± 1.21^a	15.5 ± 2.89^b	$13.8 \pm 2.12^{a,b}$	14.8 ± 2.24^b
T_M (g)	4.38 ± 1.62^a	7.55 ± 3.43^b	$5.84 \pm 2.39^{a,b}$	6.34 ± 2.37^b
S_M (g)	4.36 ± 1.61^a	7.46 ± 3.43^b	$5.77 \pm 2.34^{a,b}$	6.13 ± 2.31^b
K (T_M / T_L^3) 100	2.34 ± 0.61^a	1.94 ± 0.37^b	$2.17 \pm 0.33^{a,b}$	$1.91 \pm 0.37^{a,b}$
RI (T_M / T_L)	0.35 ± 0.09^a	0.47 ± 0.14^b	$0.41 \pm 0.11^{a,b}$	$0.42 \pm 0.11^{a,b}$

Note: Data are shown as means \pm standard deviation ($n = 82$). Descriptive variables are as follows: total length (T_L), total body mass (T_M), somatic mass (S_M), Fulton's condition (K), and body ratio index (RI). Letters indicate the statistical differences among sampling sites, two columns with the same letter do not differ (post-hoc comparison $P < 0.05$).

Effect of predation on morphological variation

Overall, I found a significant effect of predation risk on morphological traits, with fish from the low predation risk sites showing larger body traits than fish from the high predation risk sites (Tables 2.1 and 2.2). Specifically, fish from low predation sites were 15.4% longer than fish from the two high predation sites. Fish from the low predation risk sites were also about 25% heavier (total body mass and somatic mass) than fish from high predation risk sites. Furthermore, when looking at the Fulton's condition factor alone, I found that fish from the high predation risk sites displayed higher K values (16.6%) than fish from low predation risk sites; however, this trend was inversed for the body Ratio Index, which decreased by 6% in fish from high predation risk sites.

Table 2.2. Analysis of Variance results examining the effect of predation risk on morphological traits in *B. occidentalis* in Panamá.

Main Effects	d.f.	F	P
T _L (cm)	1	17.1	<0.0001*
Error	81		
T _M (g)	1	8.91	0.004*
Error	81		
S _M (g)	1	7.74	0.007*
Error	81		
K (T _M / T _L ³) 100	1	13.7	0.0004*
Error	81		
RI (T _M / T _L)	1	6.01	0.016*
Error	81		

Note: Descriptive variables are as follows: total length (T_L), total body mass (T_M), somatic mass (S_M), Fulton's condition (K), Body ratio index (RI). Asterisks show a significant effect of predation on observed morphological trait (P < 0.05).

Brain mass variation

Predation risk, drainage, and sex category had significant effects on absolute brain mass (Table 2.3). Specifically, fish from low predation risk sites had brains that were 22.8% heavier than those from high predation risk sites. Additionally, fish from the Chagres drainage had

brains that were 39.3% heavier than fish in the Bayano drainage. Overall, male brains were on average 27.4% heavier than female brains, and 59% heavier than juvenile brains. I also found significant interactions between drainage and predation risk, as well as between drainage and sex category (Table 2.3). Because of these interactions, I performed further analyses to evaluate the nature of these differences within each drainage separately.

Table 2.3. Results of the 2x2x3 factorial Analysis of Variance (ANOVA) investigating the effects of drainage, predation and sex category on brain mass variation in *B. occidentalis* of Panamá.

Main Effects	d.f.	F	P
Predation	1	5.29	0.024*
Drainage	1	16.8	0.0001*
Sex category	2	4.50	0.014*
Predation*Drainage	2	11.2	0.001*
Predation*Sex category	1	1.74	0.191
Drainage*Sex category	2	6.18	0.003*
Predation*Drainage*Sex category	1	1.14	0.288
Error	72		

Note: Significant values are denoted by an asterisk.

Within Bayano drainage, I observed a significant effect of predation risk on brain mass (Figure 2.1), with fish from the low predation risk site, Tumagantí, showing heavier brains 46.4% than in fish from the high predation risk site, Tapagrilla ($F_{1,35} = 19.0$; $P = 0.0001$). However, in Chagres, I did not observe a significant difference in brain size associated with predation risk ($F_{1,45} = 0.07$; $P = 0.41$). Finally, the stepwise analysis showed that fish somatic mass within each of the drainages explained up to 67% of the variation in brain mass ($F_{1,35} = 67.2$; $P < 0.0001$ for Bayano; $F_{1,45} = 88.3$; $P < 0.0001$ for Chagres).

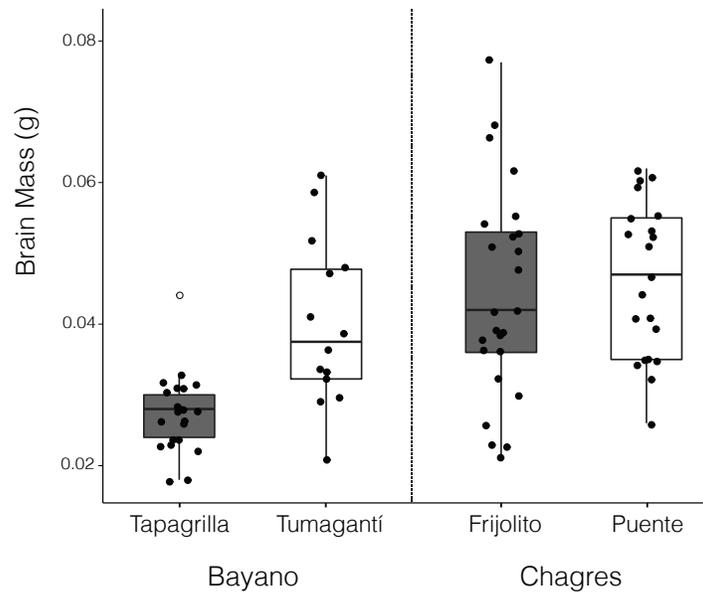


Figure 2.1. Brain mass variation in *Brachyhypopomus occidentalis* populations from high and low predation risk sites within Bayano and Chagres. High predation risk fish ($n = 47$ fish) are indicated by the dark gray boxplots, whilst low predation risk fish ($n = 35$ fish) are in white. The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values respectively, while the black dots indicate individual values. The open dots represent outliers.

I also observed a significant effect of sex category on brain mass in Bayano, but not in Chagres (Figure 2.2). Specifically, in Bayano, males had brains that were 51% heavier than females ($P = 0.002$), and 60.5% heavier than juveniles ($P = 0.006$). Nevertheless, I observed no significant difference in brain mass between females and juveniles ($P = 0.95$) within this drainage. Within Chagres (Figure 2.3b), juveniles had lighter brains than males ($P = 0.002$) and females ($P = 0.006$), but I observed no significant difference brain mass between males and females ($P = 0.59$).

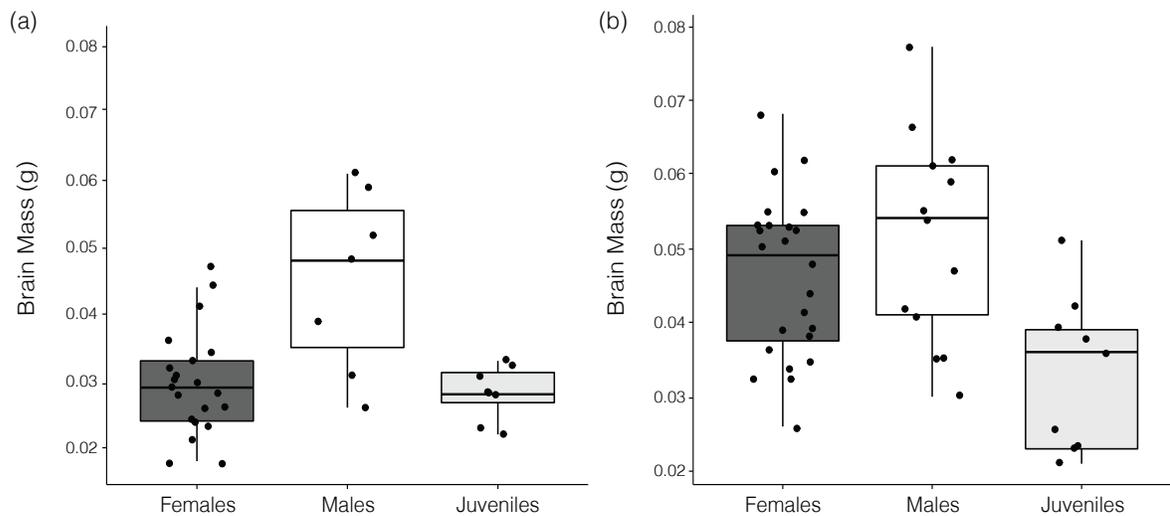


Figure 2.2. Sex based variation in brain mass in *Brachyhypopomus occidentalis* populations from (a) Bayano ($n = 36$ fish) and (b) Chagres ($n = 46$ fish). The dark grey box plots indicate female fish, the white boxplots indicate male fish while the light grey boxplots indicate the juvenile fish. The boxes indicate the interquartile range. The bold black line in the box represents the median value of the data. The upper and lower whiskers indicate the highest and lowest sample values, respectively, while the black dots indicate individual values.

Brain and body mass allometry

Across populations and independently of drainage, brain mass was positively correlated with body mass ($F_{1,82} = 119.0$; $P < 0.0001$). This correlation was also significant for high ($F_{1,46} = 56.3$; $P < 0.0001$), and low predation risk populations ($F_{1,36} = 55.7$; $P < 0.0001$) across sites. The high predation site showed a slightly steeper slope than the low predation risk site, but this difference was marginally non-significant ($t_{78} = 1.18$; $P = 0.08$; Figure 2.3).

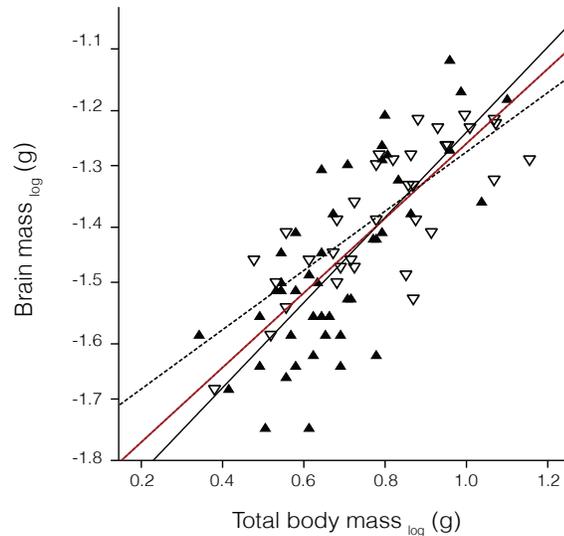


Figure 2.3. Brain-body mass allometric scaling in *Brachyhypopomus occidentalis* populations facing differing predatory pressures ($n = 82$ fish). Open triangles (dotted regression line) indicate low predation risk populations ($y = 0.508x - 1.779$; $R^2 = 0.63$); filled triangles (black regression line) indicate high predation risk populations ($y = 0.73x - 1.967$; $R^2 = 0.56$). The red regression line represents the overall ontogenetic regression ($y = 0.64x - 1.897$; $R^2 = 0.60$).

In both drainages, the ontogenetic allometric regressions were significant (Bayano: $F_{1,35} = 67.0$; $P < 0.0001$, Chagres: $F_{1,47} = 94.3$; $P < 0.0001$). However, I observed no significant difference between the drainage ontogenetic slopes ($t_{78} = 1.66$; $P = 0.102$ Figure 2.4).

When looking at allometric variation within each drainage (Figure 2.5), I found significant ontogenetic slopes for the high and low predation risk populations (Bayano high: $F_{1,22} = 6.10$; $P = 0.023$; Bayano low: $F_{1,14} = 40.4$; $P < 0.0001$; and Chagres high: $F_{1,25} = 41.3$; $P < 0.0001$; Chagres low: $F_{1,21} = 67.6$; $P < 0.0001$). However, the differences between slopes in both drainages were not significant (Bayano: $P = 0.29$; Chagres: $P = 0.36$).

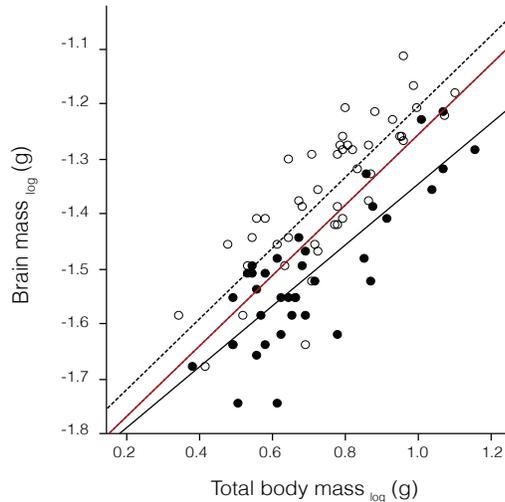


Figure 2.4. Variation in brain-body scaling relationships in *Brachyhypopomus occidentalis* populations of the two studied drainages ($n = 82$ fish). Filled circles (black regression line) indicate the Bayano populations ($y = 0.553x - 1.900$; $R^2 = 0.66$); open circles (dotted regression line) indicate the Chagres populations ($y = 0.643x - 1.849$; $R^2 = 0.68$). The red regression line represents the overall ontogenetic regression of both populations ($y = 0.64x - 1.897$; $R^2 = 0.60$).

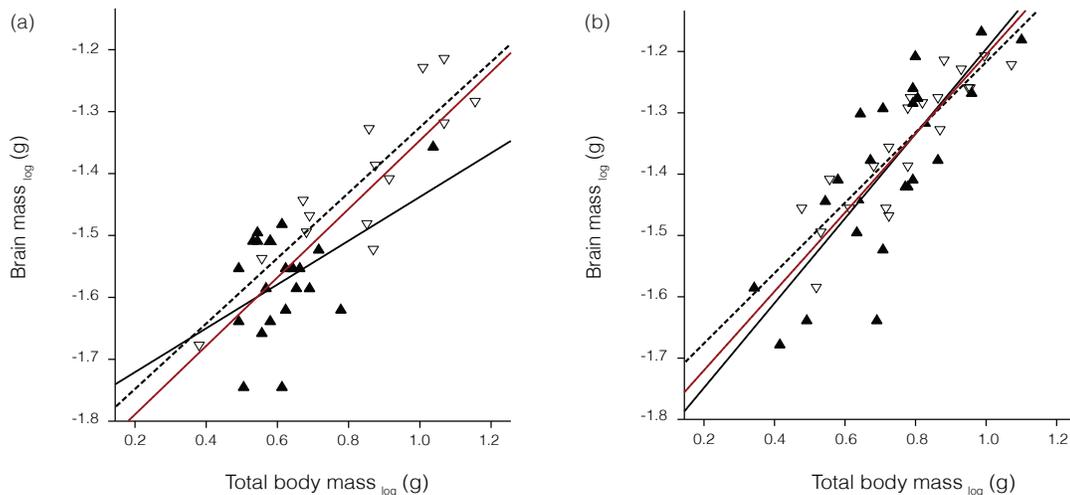


Figure 2.5. Variation in brain-body mass allometric scaling in *Brachyhypopomus occidentalis* populations facing differing predation risks within (a) Bayano ($n = 36$ fish) and (b) Chagres ($n = 46$ fish) drainages. Open triangles (dotted regression line) indicate the low predation populations (Bayano: $y = 0.528x - 1.854$; $R^2 = 0.77$; and Chagres: $y = 0.573x - 1.791$; $R^2 = 0.78$). Filled triangles (black regression line) indicate the high predation risk populations (Bayano: $y = 0.354x - 1.792$; $R^2 = 0.23$; and Chagres: $y = 0.691x - 1.887$; $R^2 = 0.64$). The red regression line represents the overall ontogenetic allometry for each drainage as follows: Bayano: $y = 0.553x - 1.900$; $R^2 = 0.66$; and Chagres: $y = 0.643x - 1.849$; $R^2 = 0.68$.

For sex and juvenile categories, the ontogenetic allometric regressions were significant (Figure 2.6; males: $F_{1,20} = 29.5$; $P < 0.0001$; females: $F_{1,45} = 44.8$, $P < 0.0001$; and juveniles: $F_{1,17} = 7.09$, $P = 0.018$). However, I did not find significant differences between males and females ($P = 0.26$), males and juveniles ($P = 0.89$) or females and juveniles ($P = 0.58$).

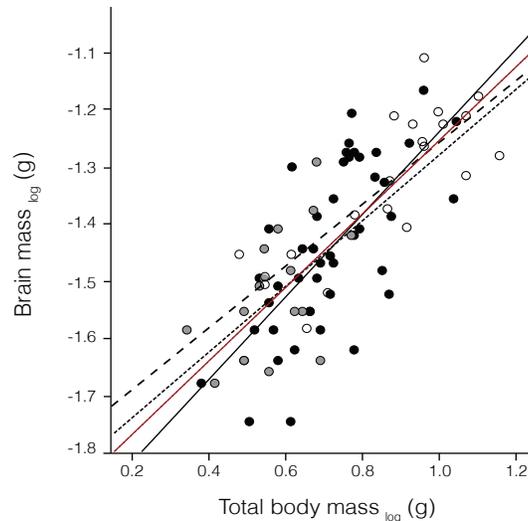


Figure 2.6. Sex-based variation in brain-body mass allometric scaling in *Brachyhypopomus occidentalis* populations ($n = 82$ fish). Open circles (dashed regression line) indicate males ($y = 0.538x - 1.798$; $R^2 = 0.62$), black circles (black regression line) represent the females ($y = 0.719x - 1.960$; $R^2 = 0.51$), while grey circles (dotted regression line) indicate the juveniles ($y = 0.572x - 1.851$; $R^2 = 0.32$). The red regression line is the ontogenetic allometry of the overall population ($y = 0.64x - 1.087$; $R^2 = 0.60$).

Within each drainage, the ontogenetic allometries were significant for both males (Figure 2.7; Bayano: $F_{1,7} = 17.5$; $P = 0.009$; and Chagres: $F_{1,13} = 25.6$; $P = 0.0003$) and females (Bayano: $F_{1,21} = 21.7$; $P = 0.0002$, and Chagres: $F_{1,24} = 37.9$, $P < 0.0001$), but not for juveniles (Bayano: $P = 0.47$ and Chagres: $P = 0.08$). Overall, the differences between slopes within each drainage were non-significant ($P > 0.05$).

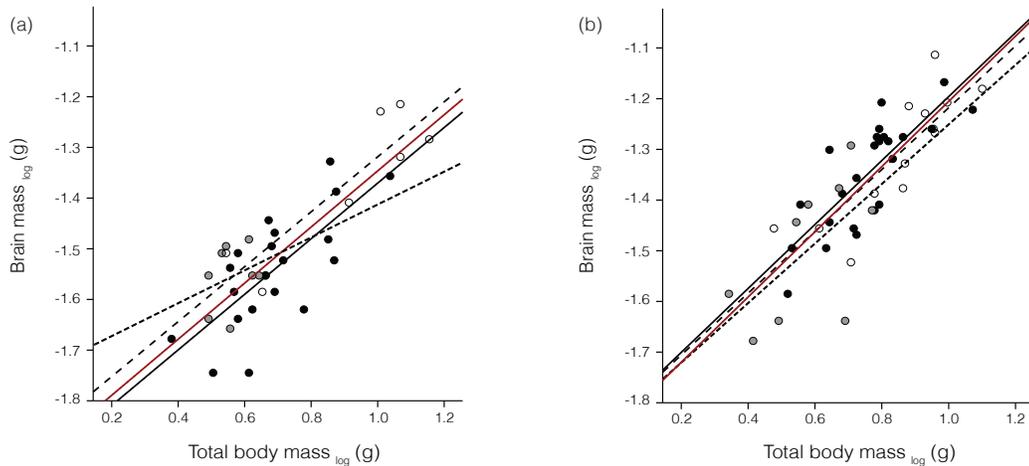


Figure 2.7. Variation in overall brain-body scaling relationships of *Brachyhypopomus occidentalis* sex categories within (a) Bayano (b) Chagres. Open circles (dashed regression line) indicate males (Bayano: $y = 0.542x - 1.861$; $R^2 = 0.78$; and Chagres: $y = 0.610x - 1.828$; $R^2 = 0.70$), black circles (black regression line) represent females (Bayano: $y = 0.548x - 1.919$; $R^2 = 0.53$; and Chagres: $y = 0.631x - 1.827$; $R^2 = 0.63$), and grey circles (dotted regression line) indicate juvenile fish (Bayano: $y = 0.324x - 1.737$; $R^2 = 0.09$; and Chagres: $y = 0.587x - 1.838$; $R^2 = 0.37$). The red regression line represents the overall ontogenetic allometry as follows: Bayano: $y = 0.553x - 1.900$; $R^2 = 0.66$; and for Chagres: $y = 0.643x - 1.849$; $R^2 = 0.68$.

Finally, when comparing the brain ontogenetic slopes between predation risk sites within each drainage to the combined population slope (Figure 2.8), I did not find significant differences ($P > 0.05$). Nevertheless, Tapagrilla populations displayed a hypoallometric slope compared to the overall population, and this observed difference was marginally non-significant ($P = 0.056$), indicating that individuals from this high predation risk site had smaller brains for their body sizes.

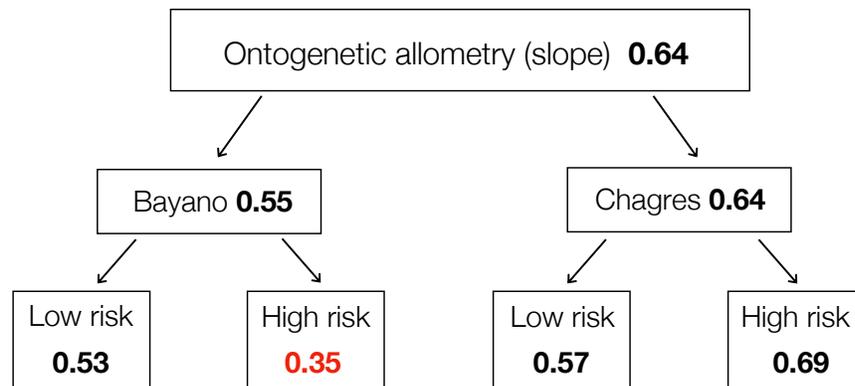


Figure 2.8. Visual representation and breakdown of the ontogenetic slopes of the studied *B. occidentalis* populations. High predation risk population within Bayano shows the most hypoallometric slope.

DISCUSSION

Overall, I found differences in brain mass in natural populations of *Brachyhypopomus occidentalis* based on predation risk and drainage. Individuals under low predation risk were larger in body size (length and mass) and were in better body condition. Although Fulton's condition factor (K) is a widely used condition index in fish (Stevenson and Woods, 2006; Datta *et al.*, 2013), *B. occidentalis* has a naturally elongated body shape, with mature males and females being longer, and consequently heavier (Hagedorn, 1988). Thus, healthier *B. occidentalis* would seem of a poorer condition, as the cube of length is used to calculate K. Fish condition is an important component of performance, competition, reproductive success and survival, and has been used by ecologists under the assumption that it reflects an individual's energetic state and overall quality (Lecren, 1951; Neff and Cargnelli, 2004; Falk *et al.*, 2017). The RI index observed in low predation risk populations could be due differences in food availability, interspecific competition, better food quality and increased foraging within those sites (Lloret and Planes, 2003). Evidence suggests that fish of better condition (higher energy

reserves), may have higher chances of survival than small fish (Adams, 1999; Shulman and Love, 1999). This is particularly evident during nonfeeding periods, when fish in poorer condition could be more susceptible to external stressors, such as predation, parasites, temperature variation and contaminants (Lloret and Planes, 2003).

Individuals facing low predation risk also had brains that were heavier than those from high predation risk sites, independently of drainage. Furthermore, the brain – body mass ontogenetic allometric scaling showed a significant effect of predation risk, with the high predation risk population of *Tapagrilla* showing a hypoallometric slope compared to the low predation risk populations. These results suggest that phenotypic plasticity might be driving the observed variation, as adaptive phenotypic plasticity allows organisms to change or adjust morphologically, physiologically or behaviourally in response to environmental stimuli, without altering their genomes (West-Eberhard, 1989; Gonda *et al.*, 2011; 2013; Salvanes *et al.*, 2013; White and Brown, 2015; Kotschal *et al.*, 2017).

The role of predation risk in brain mass variation

Overall, I found that predation risk had a significant effect on brain mass variation in *B. occidentalis*. Specifically, my finding of smaller brain mass at high predation risk sites is consistent with previous studies on *B. occidentalis* that found a lower density of proliferating cells in the telencephalon (Dunlap *et al.*, 2016) at the same high predation sites, suggesting that predation risk may indeed be a driver for brain mass variation. Other gymnotiform electric fish, such as *B. gauderio* and *A. leptorhynchus*, also displayed inhibition of brain cell proliferation in high predation risk sites (Dunlap *et al.*, 2017). Similarly, research on male killifish (*Rivulus hartii*) showed that individuals exposed to predators displayed smaller brains than their counterparts in environments without predators (Walsh *et al.*, 2016). It has been suggested that

inhibition of brain cell proliferation may solicit behavioural changes that make fish more vulnerable to predators or other environmental challenges (Dunlap *et al.*, 2016), particularly in areas of the brain that involved in predator risk assessment such as the telencephalon (Portavella *et al.*, 2004; Dunlap *et al.*, 2017). The observed reduction in brain mass could also be attributed to decreased foraging, which could in turn reduce the overall energy allocation to brain development and maintenance (Eifert *et al.*, 2015; Axelrod *et al.*, 2020).

Smaller brains in the context of predation could result from trade-offs associated with brain size development and maintenance. For instance, fish in general have constant energetic costs related to neurogenesis during adulthood, as well as brain maintenance and brain development (Zupanc, 2001; Charvet and Striedter, 2011). In knifefish, the cerebellum is associated with electric signal processing and interpretation (Bell and Maler, 2005a; Sukhum *et al.*, 2018; Sukhum *et al.*, 2019), and is exceptionally large when compared to other brain regions (Nilsson, 1996; Butler and Hodos, 2005; Sukhum *et al.*, 2018). In addition, *B. occidentalis* coexist with a predatory catfish, which similar to gymnotiforms, possess ampullary receptors capable of detecting low-frequency spectral components of the electric signals (Stoddard and Markham, 2008). This detection of weakly electric fish by electro-sensing predators is known as “eavesdropping” (Stoddard *et al.*, 2019), and it can lead to a shift of the fish’s electric signal out of the sensory range of predators (Hanika and Kramer, 2000; Stoddard and Markham, 2008). This cloaking mechanism has been demonstrated in *B. occidentalis* under high predation risk from the electro-sensing catfish *Rhamdia quelen*, where *B. occidentalis* emits lower frequency of EOD peak-power (Tran, 2014). Nevertheless, this shift in the EODs can be energetically costly (Salazar *et al.*, 2013; Stoddard *et al.*, 2019). In Gymnotiformes, oxygen consumption related to EOD production is around 30% of daily energy intake (Salazar *et al.*, 2013; Crampton, 2019), and male knifefish budget about 11-22% of the daily energy intake to

electric signalling alone (Salazar and Stoddard, 2008). Large brains can also contribute to high oxygen consumption rates in electric fish species (Nilsson, 1996). If the oxygen consumption rates of *B. occidentalis* are similar to those reported in *B. gauderio* (Salazar and Stoddard, 2008), I suggest that there might be trade-offs between EOD production and brain size, with smaller brains resulting from the increased metabolic cost associated with signal cloaking. The “mosaic evolution” hypothesis proposes that selective pressures act on specific brain regions independently, resulting in changes in the relative sizes of those specific areas (Barton and Harvey, 2000; Eifert *et al.*, 2015). If the brain in *B. occidentalis* follows the “mosaic evolution” theory, we should expect an enlargement of the cerebellum, as this would allow individuals to accommodate the costs of this anti-predator mechanism, whilst supporting the areas of the brains that are associated with signal modulation and interpretation. However, future studies are required to test whether this is true in *B. occidentalis*.

Although my research found that smaller brains prevail in environments with high predation risk, this does not translate into larger brains being unfavourable under high predatory pressure, but rather that this pressure may impose ecological constraints on brain mass. For example, female guppies from high predator sites have relatively larger telencephalon sizes and larger overall brains compared to the males (Kotrschal *et al.*, 2017). Moreover, large-brained female guppies participate less frequently in predator inspections and are able to make a quick assessment of the predation risk (van der Bijl *et al.*, 2015). Although larger brains seem to be favoured by selection, this does not seem to be the case for *B. occidentalis*, at least in some environments. Hence, future studies should explore the contribution of brain size to anti-predator behaviour in both males and females in *B. occidentalis*. Also, it would be interesting to explore whether there is a link between large brains and the capacity individuals to make more accurate assessments about a perceived threat.

Drainage effects on brain mass variation

In my study, significant differences in brain mass were observed between the two drainages. Fish from the Chagres drainage exhibited brains that were significantly heavier than those from fish from Bayano. When comparing within-drainage brain mass variation, no difference was observed between sites in Chagres; while in Bayano, the fish from Tumagantí had brains that were significantly heavier than fish from Tapagrilla. A recent study on *B. occidentalis* populations from the same drainages has reported differences in EOD waveforms based on geographic distances, and correlated geographic distances to genetic distances (Picq *et al.*, 2016), suggesting that the differences observed between these populations might be a result of potential genetic differences associated with the different lineages (Picq *et al.*, 2014). Nevertheless, the significant differences within the Bayano drainage suggest that the observed variation in brain mass could also be a result of the cumulative effect of various selective forces within each drainage.

Defining what drainage differences actually cause the observed variation in brain mass leads to major questions; specifically, whether factors other than geography act as confounding sources of variation. In fact, evidence suggests that in addition to strict geographic isolation (Aguilar *et al.*, 2019), local ecological factors may drive variation in brain size. One such factor to consider would be the differences in environmental complexity within the two independent drainages, and between sampling sites. Although this was outside of the scope of my study, environmental complexity has been shown to affect brain size variation in fish (Salvanes *et al.*, 2013; Liao *et al.*, 2015; White and Brown, 2015; Axelrod *et al.*, 2018; Fong *et al.*, 2019). A comparative study of four goby species found that fish with larger brain volumes were found in rock pool-dwelling populations in contrast to populations living in less complex sandy environments (White and Brown, 2015). Changes in the overall environmental complexity

could also affect food availability, requiring individuals to use different foraging strategies, which could require higher brain functions, and larger brains (Markham *et al.*, 2016). This is especially important to consider with electric fish species that use EODs to navigate (Alves-Gomes *et al.*, 1995). Knifefishes use active electrosensing, a mechanism by which variations in the electric field generated determine the presence of objects surrounding the fish (Stoddard *et al.*, 1999; Markham *et al.*, 2016). In my study, Tumagantí river seems more structurally complex, with its riverbed made of varying sediments, large boulders and root masses, while Tapagrilla site is a stream, and its bed is made of gravel and sand. Also, *B. occidentalis* in Tumagantí were always hidden within dense mature tree roots in the water, between boulders as opposed to under decomposing organic matter in Tapagrilla. Although these parameters were not quantified, I suggest that increased habitat complexity would require *B. occidentalis* to create more sophisticated spatial maps in order to navigate and acquire food with possible consequences for variation in brain mass.

Another potential factor affecting brain mass variation is social interaction. Knifefishes greatly depend on intraspecific and interspecific communication in order to thrive, thus placing an importance on social hierarchy and complexity (Hagedorn, 1988; Picq *et al.*, 2016). During sampling in Tumagantí, other electric fish species were identified including *Sternopygus dariensis*, *Apteronotus rostratus*, and *Eigenmannia* sp., while in Tapagrilla, *B. occidentalis* was the only electric fish encountered. These observations show that *B. occidentalis* from Tumagantí lives in a richer electric fish community, where they not only have to participate in active electro-sensing with conspecifics during aggressive or courtship interactions (Hagedorn and Carr, 1985), but also with other electric fish species, which could result in the observed larger brains at this site. Recent studies have linked fish brain size with species richness, diversity and abundance, attributing the number of species present or number of individuals to

social complexity (Shumway, 2008; 2010). Under natural conditions, if the fish group size increases, the number of possible social cues and responses should also increase (Bshary *et al.*, 2014). Evidence suggests that differences in social complexity contribute to variation to social learning, dominance, competition for mates, and potentially reproductive activity (Gonzalez-Voyer *et al.*, 2009; Clutton-Brock and Huchard, 2013). In Lake Tanganyika cichlids, social complexity has dramatic effects on brain size variation, where the telencephalon size and the cerebellar size were positively correlated with increased species interactions (Pollen *et al.*, 2007).

In this study, I also found that females in Chagres had heavier brains than females in Bayano. Females with larger brains could benefit from the cognitive advantages of higher encephalization (van der Bijl *et al.*, 2015; Kotrschal *et al.*, 2017). In electric fishes, it has been suggested that the mating system is based on female choice (Hagedorn and Carr, 1985; Stoddard *et al.*, 1999), a task that may favor females with larger brains. Larger brains in females have also been associated with increased survivability and predator evasion (Kotrschal *et al.*, 2015a; van der Bijl *et al.*, 2015). However, Bayano females exhibited smaller brains than Chagres females, and also had brains that were significantly smaller than males. I suggest that within that drainage, individuals might be subjected to different selective pressures that may favor smaller brain sizes overall, and specifically in females. On the other hand, males had the largest brains in both sampling sites. This suggests that males and females could be facing different cognitive demands, associated with a difference in sex specific signal production and behaviours, including balancing signal cloaking under predation risk, aggressive interactions between males and courtship interactions with females (Hagedorn and Carr, 1985; Curtis and Stoddard, 2003; Salazar and Stoddard, 2008; Stoddard and Markham, 2008; Buechel *et al.*, 2019; Stoddard *et al.*, 2019).

Brain mass and body mass allometry

Teleost fishes exhibit life-long neurogenesis and continuous body growth (Leyhausen *et al.*, 1987; Kaslin *et al.*, 2008; Eifert *et al.*, 2015; Salas *et al.*, 2015; Lisney *et al.*, 2017; Axelrod *et al.*, 2020). Thus, shifts in brain size might come at the expense of other organs or metabolic activities (Aiello and Wheeler, 1995; Striedter, 2005; Kotrschal *et al.*, 2013a; Tsuboi *et al.*, 2014), leading to strong patterns of covariation between brain size, individual brain compartments and body size, also known as allometric scaling (White and Gould, 1965; Aboitiz, 1996; Montgomery *et al.*, 2016; Sukhum *et al.*, 2018). These allometric relationships suggest the presence of significant constraints limiting independent evolution of brain size, thus limiting adaptive response to selection by external ecological factors (Montgomery *et al.*, 2016). In this study, I found that somatic mass explained more than 67 % of the total observed variation in brain mass in *B. occidentalis*. This fundamental relation between brain and body mass reveals much about the constraints and adaptations that lead to evolutionary change within and among species (Jerison, 1973; Bauchot *et al.*, 1977; Tsuboi *et al.*, 2016).

Previous studies determined that the brain-body mass scaling relationships of all vertebrates (evolutionary allometry) has a mean coefficient of 0.67 (Bauchot *et al.*, 1977). I found that *B. occidentalis* populations have an overall ontogenetic allometric slope of 0.64, which is slightly higher than what has been reported for other fish living in highly complex environments (Tsuboi *et al.*, 2016). This is not surprising given the high encephalization of electric fishes (Salazar *et al.*, 2013; Sukhum *et al.*, 2018), and the use of EODs (Nilsson, 1996; Salazar and Stoddard, 2008). When comparing slopes based on predation risk, populations under high predation risk had significantly smaller brains for their body sizes compared to low predation risk populations (high predation risk: 0.73 and low predation risk: 0.51). Within Chagres, no difference in the slopes was observed between the high and low predation risk sites

(high: 0.64; low: 0.57). Interestingly in Bayano, the slope for the high predation risk site was hypoallometric compared to the low predation risk site (high: 0.35 and low: 0.53), although this difference was not significant. These differences could be attributed to the slight environmental variations between specific sampling sites, the number of fish sampled, as well as the proportion of juveniles to mature individuals within each respective site.

Although brain size can be constrained as a result of invariant growth mechanisms such as brain-body size allometry (Huxley, 1932; Jerison, 1973; Tsuboi *et al.*, 2016), recent evidence suggests that external factors might also contribute to this allometric variation (Pelabon *et al.*, 2013; Voje *et al.*, 2014; Tsuboi *et al.*, 2016; Lisney *et al.*, 2017). Collectively, my results suggest that the interaction between predation risk and geographic isolation may lead to variation in brain-body mass allometry parameters in electric fishes. If knifefish expend energy actively avoiding predators, this may lead to a reduction in foraging, and consequently resource uptake (Wagner *et al.*, 2009; Sullam *et al.*, 2015). Under continued threat, such a reduction in resource uptake would result in the limited energy reserves being re-allocated to EOD production to keep evading predators, potentially inducing fitness trade-offs between EOD signalling and body growth (Peckarsky and McIntosh, 1998; Walsh *et al.*, 2016; Dunlap *et al.*, 2019). Thus, my results suggest that fish under perceived stressful conditions may not be favoring the development of neural tissue, or even overall growth, but rather using that energy for other expensive activities such as predator avoidance and electric signal modulation in the face of predation risk, as previously demonstrated under laboratory conditions (Stoddard *et al.*, 2019); however, this requires further investigation.

Conclusion

The observed variation in brain mass among populations of *B. occidentalis* can be attributed to a multifactor response of the knifefish to differences in environmental complexity, social interaction and predation regimes. Although I used sites that have previously been identified by other researchers as low or high predation risk sites (Picq *et al.*, 2014; Stoddard *et al.*, 2019), I acknowledge that predation risk alone may not account for the observed variation in brain mass. Thus, future studies should focus on disentangling the contribution of individual factors such as differences in habitat structure, social complexity and dissolved oxygen content among others, to the overall brain mass variation. In addition, it would be beneficial to perform this study at a wider scale by including more sampling sites and individuals, but also see if the results can be replicated in captivity, where additional parameters can be controlled and analyzed (such as food availability, social interaction and cognitive tests). It would also be interesting to investigate the overall energy expenditure of knifefish during EOD modulation under varying predation risk. Finally, because of the relative importance of the cerebellum in processing and modulating EODs in weakly electric fishes, it is important that future studies investigate whether the cerebellum is as plastic as the telencephalon, as suggested in other studies. Furthermore, we should investigate if variation in cerebellum size can be linked to a variation in EOD frequency and amplitude, and whether variation exists during ontogeny.

GENERAL SYNTHESIS AND DISCUSSION

GENERAL SYNTHESIS

The main goal of my thesis was to explore the intraspecific variation in life history traits of the electric fish *Brachyhypopomus occidentalis* under natural conditions, and to determine whether ecological factors, such as predation risk and geographical isolation by drainage, contribute to variation in brain mass and reproductive output. In Chapter I, I described the reproductive biology of mature *B. occidentalis* and explored how predation risk can affect reproductive traits such as gonad mass, fecundity and gamete size in both females and males. Previous studies have shown that the physiological stress response in animals is an adaptive mechanism geared towards increased survival, resulting in limited resources allocated to other essential functions (Hawlena and Schmitz, 2010; Clinchy *et al.*, 2013). Therefore, fish under stressful conditions (such as increased predation risk) may reduce their reproductive output by investing less energy into gonad development, which results in reduced fecundity and smaller gamete sizes (Schreck *et al.*, 2001; Billman *et al.*, 2011). In high predation risk sites, female *B. occidentalis* had smaller ovaries, lower absolute and relative fecundities, and fewer oocytes per maturity stage, strongly suggesting that fish undergo energy investment trade-offs under stressful conditions. Interestingly, female oocyte traits did not vary under predation risk, but did vary based on drainage, suggesting that other factors such as food availability, water quality, temperature and habitat structure, might be contributing to oocyte size variation in this species. Future studies should investigate variation in resource availability, nutrient quality and quantity, and stomach contents in natural populations, to determine whether the observed reduction in reproductive output in females is a result of reduced nutrient intake rather than actual physiological stress induced by increased predation risk.

On the other hand, male reproductive traits (testes mass and sperm morphometry traits) did not vary based on predation risk or drainage. This could be due to small sample sizes or low replicates for both drainage and predation risk sites. Hence, future studies should look into a wider sampling range across more sites to test whether these two ecological factors are involved in male reproductive trait variation in this species. Future studies should also consider exploring other ecological factors, such as increased intra and inter-specific competition, to test whether variation in social complexity contributes to gonad and sperm trait variation in male *B. occidentalis*.

In Chapter II, I explored brain mass variation in *B. occidentalis* populations, and brain-body ontogenetic allometry, under varying predation risk. Gymnotiforms are highly encephalized, and studies have attributed this to the complex electric signal processing and interpretation capacity of the brain (Nilsson, 1996; Salazar *et al.*, 2013; Sukhum *et al.*, 2018). I found that variation in brain mass was associated with predation risk, drainage and sex, with fish from high predation risk sites showing significantly lighter brains. Brain-body ontogenetic allometry also varied based on predation risk, although the observed differences were not statistically significant. My results suggest that predation risk is an important driver of brain mass variation in electric fishes; however, its effect can vary with sex, developmental stage and drainage. Future studies should examine whether the smaller brain mass observed in high predation risk populations are a result of reduced nutrient consumption and foraging, in response to increased predation risk. Finally, studies should explore the potential implications of smaller brains on electric signal production and survival in *B. occidentalis*.

Overall, my thesis highlights predation risk as a key selective factor in both reproductive trait and brain mass variation. However, the effect of predation can vary based on sex and geographical isolation; and the observed variations can be attributed to multifactor responses to

differences in resource availability, environmental complexity and individual constraints, although how these factors are synergistic in nature requires further investigation. Furthermore, we still do not know to what extent the energy demands required for brain maintenance are balanced with the energy required for reproduction in *B. occidentalis*.

The Expensive-Tissue Hypothesis (ETH) and “Energy Trade-off Hypothesis” state that the metabolic requirement of large brains is offset by a corresponding reduction of other costly tissues and processes (Aiello and Wheeler, 1995; Isler and van Schaik, 2006b; Isler and van Schaik, 2009; Tsuboi *et al.*, 2015). As such, if these hypothetical models apply to *B. occidentalis* individuals, I should expect to see a trade-off between large brains and reproduction, resulting in organisms with lower reproductive output. In this general synthesis, I tied the two chapters of my thesis together by exploring in the potential correlation between brain mass and reproductive effort in the studied *B. occidentalis* populations. For this final part of my research, I was interested in determining whether trade-offs between these life history traits existed, and whether they varied based on predation risk within in this species.

MATERIALS AND METHODS

Only mature fish processed in the previous chapters were used in this synthesis chapter. Please refer to “Ovary and oocyte analysis” and “Testicle and sperm analysis” in Chapter I; and “Brain processing” in Chapter II for more information on data collection.

STATISTICAL ANALYSES

I analyzed all of the data using IBM SPSS Statistics 24. All analyses were conducted separately for females and males. I started by performing stepwise regression analyses to determine which factors best explain the observed variation in brain mass, including gonad traits for both sexes,

along with oocyte traits for females, and sperm morphology traits for males. Oocyte traits and sperm traits were analyzed for possible collinearity using a principal component analysis (PCA). To reduce the number of variables and data redundancy, I retained the gamete traits with the highest Eigenvalues while the gamete traits with lower Eigenvalues were excluded. I then performed Pearson correlations between brain mass and the reproductive traits obtained by the stepwise regressions separately for high predation and low predation risk populations, to explore trade-offs in energy allocation for brain maintenance and reproductive traits. The data for brain mass and gonad mass and gamete size traits were corrected for body mass by using the residuals from the general linear models (Walsh *et al.*, 2016).

RESULTS

For females, the best predictor for brain mass variation was ovary weight (Table 3.1). A second stepwise regression analysis with only oocyte traits revealed that mature oocyte volume best explained variation in brain mass. For males, testes mass best predicted brain mass variation (Table 3.1). When testing for sperm traits, the stepwise regression revealed that hydrodynamic ratio best explained brain mass variation.

Overall, I observed positive correlations between female brain mass and female reproductive traits (Figure 3.1), however, none of the trait correlations in females varied significantly between predation risk and drainage populations ($P > 0.05$). Brain mass and male reproductive traits were negatively correlated in high predation risk sites but positive in low predation risk sites, although the associations were non-significant (Figure 3.2). Again, I observed no differences in male trait correlations between drainages ($P > 0.05$).

Table 3.1. Stepwise regression analysis of brain mass variation in *B. occidentalis* females and males.

Females	β	Slope	r^2	F	P
Ovary mass	-1.227	0.207	0.457	30.3	< 0.0001
Mature oocyte volume	-1.354	0.266	0.189	7.40	0.043
Males	β	Slope	r^2	F	P
Testes mass	0.461	0.461	0.608	26.4	< 0.0001
Hydrodynamic ratio	-8.191	-2.586	0.338	7.15	0.018

Note: Female ovary mass (n = 37 fish), female mature oocyte volume (n = 21 fish), male testes mass (n = 19 fish), male hydrodynamic ratio (n = 15 fish).

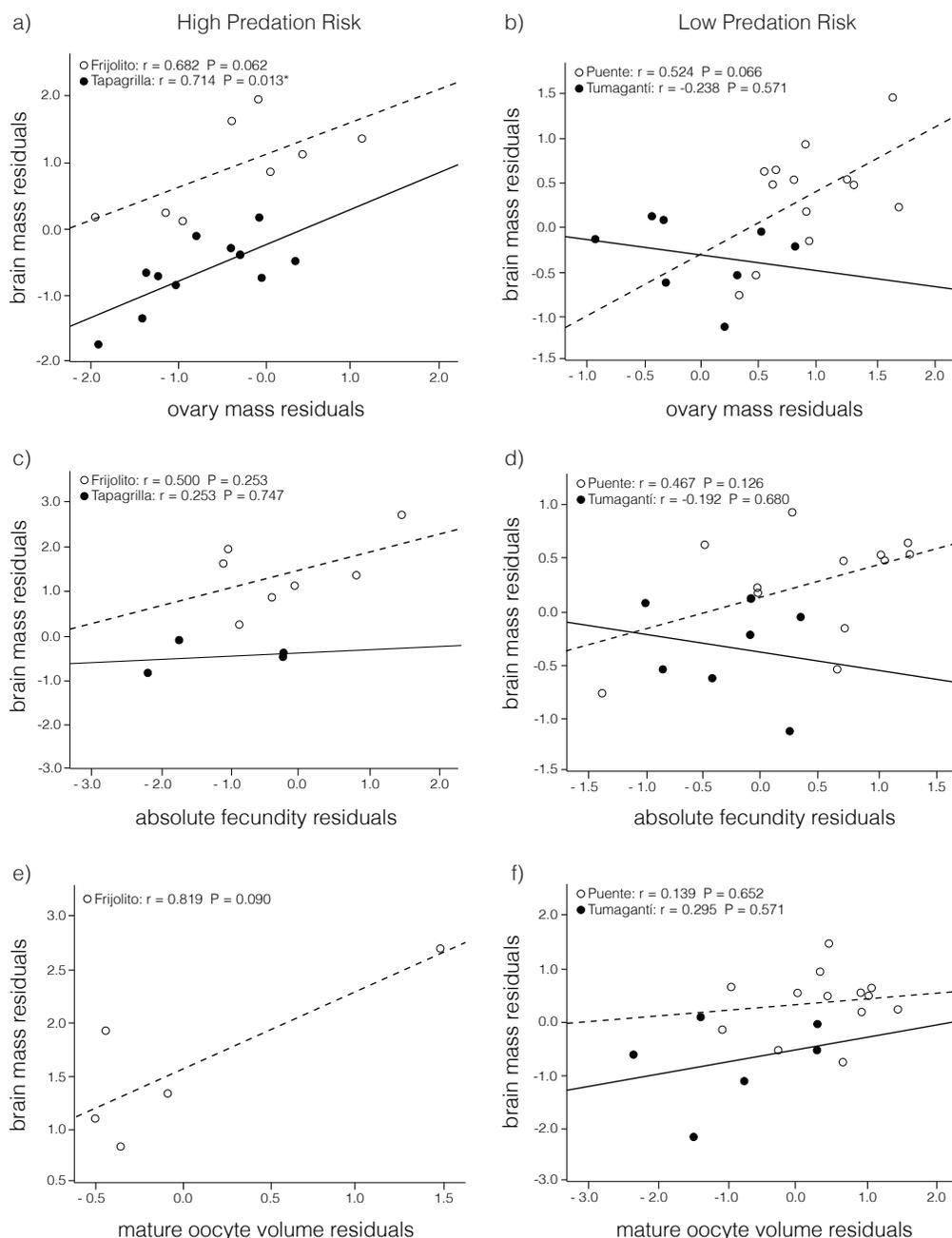


Figure 3.1. Correlations between female brain mass and ovary mass, absolute fecundity and mature oocyte volume. a) Brain mass versus ovary mass for high predation females, b) brain mass versus ovary mass for low predation females, c) brain mass versus absolute fecundity for high predation females, d) brain mass versus absolute fecundity for low predation females, e) brain mass versus mature oocyte volume for low predation females, f) brain mass versus mature oocyte volume for low predation females. Open circles (dashed line) represent Chagres population, filled circles (solid line) represent Bayano populations. a) Bayano correlation was significant ($F_{1,10} = 9.39$; $P = 0.013$). All other correlations were non-significant ($P > 0.05$).

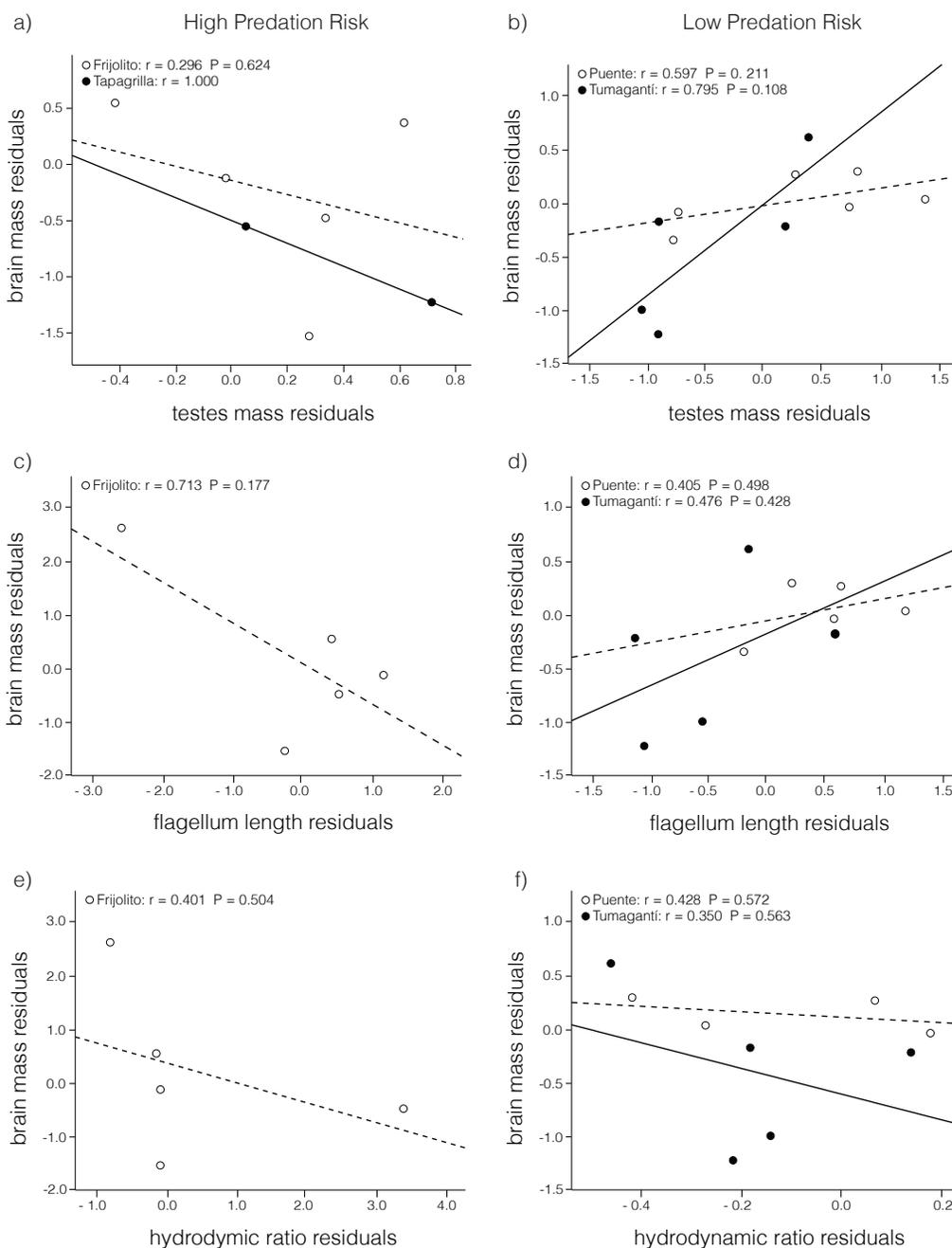


Figure 3.2. Correlations between male brain mass, testes mass, flagellum length and hydrodynamic ratio. a) brain mass versus testes mass for high predation males, b) brain mass versus testes mass for low predation males, c) brain mass versus flagellum length for high predation males, d) brain mass versus flagellum length for low predation males, e) brain mass versus hydrodynamic ratio for low predation males, f) brain mass versus hydrodynamic ratio for low predation males. Open circles (dashed line) represent Chagres population, filled circles (solid line) represent Bayano populations. All correlations were non-significant ($P > 0.05$)

GENERAL DISCUSSION

Life history theory postulates that the simultaneous optimization of different fitness correlates can be constrained by life history trade-offs (Moore *et al.*, 2016). Consequently, an increase in fitness due to selection on one trait is counteracted by variation in another trait, particularly when individuals are under stress (Schreck, 2010; Clinchy *et al.*, 2013). Evidence suggests that electric fish can be highly encephalized (Nilsson, 1996; Sukhum *et al.*, 2016; Sukhum *et al.*, 2019); thus, the metabolic and energetic demands of brain maintenance could be met by changing energy allocation in reproductive effort (Isler and van Schaik, 2006a; Tsuboi *et al.*, 2015). Large brains have been linked to increased cognitive abilities in fishes (Buechel *et al.*, 2018; Fong *et al.*, 2019), and since cognitive abilities are important to facilitate behaviors such as foraging (Hughes *et al.*, 1992; Boogert *et al.*, 2011), predator avoidance (Kotrschal *et al.*, 2015a; van der Bijl *et al.*, 2015), and mate selection (Corral-Lopez *et al.*, 2017; Corral-Lopez *et al.*, 2020), individuals with increased cognitive abilities are likely to have higher reproductive success (Sol, 2009; Boogert *et al.*, 2011). For instance, increased foraging efficiency in females would allow larger investment into individual oocytes (Kotrschal *et al.*, 2015b; Barneche *et al.*, 2018), resulting in larger oocyte volumes (Isler and van Schaik, 2009; Barton and Capellini, 2011; Tsuboi *et al.*, 2015). Larger oocytes have been linked to increased offspring growth rates (Einum and Fleming, 2000; Lasne *et al.*, 2018), swimming performance (Thorn and Morbey, 2018), and survival rates (Segers and Taborsky, 2012; Smalås *et al.*, 2017). More importantly, for highly encephalized organisms, larger oocytes translate to larger energy reserves for brain development (Tsuboi *et al.*, 2015). However, this cost, along with the cost of high encephalization, could be offset by a reduction in oocyte quantity reduced fecundity (Isler and van Schaik, 2006a; Isler and van Schaik, 2006b). There are some studies in support of the

Expensive Tissue Hypothesis (ETH) and the “Energy Trade-off Hypothesis” in fish, where females with large brains have been associated with smaller clutch sizes and reduced fecundity (Kotrschal *et al.*, 2013a; Tsuboi *et al.*, 2015). In my thesis however, *B. occidentalis* females with larger brains were associated with greater mature oocyte volumes regardless of environmental variation, supporting the hypothesis that individuals with large brains invest highly into oocyte quality (larger egg size), potentially for the production of juveniles with larger brains. Because female fish with larger brains also had higher absolute fecundity and gonad mass, my thesis does not provide evidence for the ETH in this species.

Interestingly, I did not find evidence of the ETH in *B. occidentalis* males. I observed a weak positive correlation between brain mass and testes mass, but not with other reproductive traits. However, as explained in Chapter I, this could be a reflection of the small sample sizes. Despite this, there is evidence of positive associations between male brain mass and reproductive investment in male fishes (Kotrschal *et al.*, 2015c). For example, male goldfish (*Carassius auratus*) testes mass was positively correlated to brain mass (Liu *et al.*, 2014), while large brains in male guppies (*Poecilia reticulata*) were associated with higher precopulatory sexual traits (Kotrschal *et al.*, 2015c). Individuals with larger brains might have greater cognitive abilities that are associated with increased mate attraction and reproductive success, suggesting that sexual selection could influence variation in brain mass variation, although this requires further investigation in this species (Boogert *et al.*, 2011). These findings also suggest that males may compensate for variation in brain mass without sacrificing other expensive tissues, although this is unlikely given the high costs of electric signal production (Stoddard and Salazar, 2011; Salazar *et al.*, 2013) and requires further investigation.

Moreover, I found no significant correlation between increased brain mass and sperm traits, although the trend was negative. One plausible reason for this lack of trade-offs between

brain mass and reproductive output in male *B. occidentalis* might be their low overall investment to gonad development. Evidence suggest that the cost of gonad development is greater for females than males (Parker, 1970; Hayward and Gillooly, 2011; Yong and Grober, 2014). I observed in my study that male testes mass represented 0.5% of total body mass, while ovary mass accounted for around 3% of total body mass in females. Compensation for high encephalization should therefore be expected in females as reproduction is such a costly process, but perhaps not in males of this species. Nevertheless, we also need to keep in mind that electric organ discharge (EOD) production and electric communication can incur in high energetic costs for males (Salazar and Stoddard, 2008; Salazar *et al.*, 2013). As discussed in the second chapter, knifefish males can partition an average of 22% of daily oxygen intake on electrogenesis, compared to females that budget around 3% (Salazar and Stoddard, 2008; Stoddard and Salazar, 2011; Salazar *et al.*, 2013). During the reproductive season, male knifefish generate low peak power frequency and high amplitude EODs (Crampton, 2019), enhancing their EOD waveform at night (or circadian swing) when most social interactions occur (Hagedorn, 1988; Franchina *et al.*, 2001; Salazar *et al.*, 2013). It has been observed that *B. occidentalis* constantly emit EODs for electrolocation but modulate discharge rates to produce signals for aggressive interactions between males, and courtship interactions between males and females (Hagedorn, 1988; Stoddard *et al.*, 1999). Knifefish can also modulate their EODs to avoid detectability by electrosensing predators, and evidence suggests that electric fishes pay an energetic price for the ionic mechanisms implicated in the reduction of low-frequency energy in their signals (Salazar *et al.*, 2013; Stoddard *et al.*, 2019), indicating that this shift in the EOD spectrum might also be costly. It could be possible that EOD generation comes at a greater energetic cost for male *B. occidentalis* than gonad development, forcing

trade-offs to occur between electric signalling and brain development and its maintenance in this species.

Knifefish could benefit from greater neuronal investment under high predation risk if higher encephalization resulted in sensory or cognitive benefits (Reddon *et al.*, 2018; Mitchell *et al.*, 2020). When weakly electric fishes generate an electric field, perturbations within that field are sensed by tuberous electroreceptors on the surface of the skin (Carr *et al.*, 1982), and primary afferent input from the electroreceptors are received by the electrosensory lobe (ELL), a cerebellum-like structure found in the hindbrain of electric fish (Bell *et al.*, 2008). The efferent output is received by the torus semicircularis (TS), found in the midbrain, where further neural projections to structures like the optic tectum, the valvula cerebelli, and other processing centers, are reached (Bell and Maler, 2005b; Salazar *et al.*, 2013). Both the TS and the ELL receive feedback from the cerebellum (Carr *et al.*, 1981; Bell and Maler, 2005b; Marquez *et al.*, 2013; Salazar *et al.*, 2013), indicating that the cerebellum is responsible for processing electrosensory information (Bell and Maler, 2005a; Sukhum *et al.*, 2018). The potential cognitive advantages of increased cerebellum size in *B. occidentalis* under predation risk could lead to more efficient signal interpretation (Salazar *et al.*, 2013), increased capacity for signal cloaking (Stoddard *et al.*, 2019), or even increased predator detection and avoidance by way of spatial maps (Markham *et al.*, 2016); thus, it might be worth the cost if it results in increased fitness (Mitchell *et al.*, 2020), although this requires further investigation.

Because predation risk can shape EOD generation in this species (Tran, 2014; Stoddard *et al.*, 2019), and the evolution of the cerebellum is driven by electrosensing and electric signalling (Bell *et al.*, 2008), it possible that predation risk only exerts a selective pressure on this specific brain region. As discussed in Chapter II, selective pressures can act on specific brain regions independently, resulting in changes in the relative sizes of those specific areas

(Barton and Harvey, 2000; Montgomery *et al.*, 2016; Sukhum *et al.*, 2018). This “mosaic brain hypothesis” considers both brain architecture and associated fitness-related traits and suggests that brain regions of more significance in a particular context can develop faster than less “important” or “useful” regions (Kihlslinger and Nevitt, 2006; Gonda *et al.*, 2012). As evidenced by my results, environmental factors can induce plasticity of overall brain mass, but it is also possible that predation risk acts as a selective pressure on the cerebellum, leading to a highly developed ELL at the expense of other brain regions (Barton and Harvey, 2000; Eifert *et al.*, 2015). Simultaneously, predation risk could constrain the development of the other brain regions (Portavella *et al.*, 2004; Dunlap *et al.*, 2016), contributing to the overall smaller brain observed. Finally, costs of neural tissue maintenance, reduced nutrient intake and quality, as well as increased physiological stress and shifts behaviour could all contribute to the small brain mass and low reproductive output observed in *B. occidentalis* under high predation risk, as discussed in both chapters. Future studies should determine whether all brain regions scale allometrically to overall brain size; and explore whether individual parts vary independently according to environmental stressors, as observed in other fish species (Kotrschal *et al.*, 2017; Sukhum *et al.*, 2018). Furthermore, it would be important to explore if increased brain size, particularly increased cerebellum size in *B. occidentalis*, are linked to a greater capacity for signal cloaking, and overall signal production in electric fishes. Finally, future studies should quantify the EODs to get a better idea of how electric signalling contributes to variation in trade-off strategies between brain mass and reproduction, as well as overall life history patterns in electric fishes.

Finally, contrary to my hypothesis, I did not observe differences in correlations between brain mass and reproductive output based on predation risk in either sex. The lack of significance in my results is likely due to the low sample size of mature females and males per

site, particularly in terms of oocyte and sperm traits. Generally, fishes cope with stressful conditions by allocating energy to functions linked with increased survival, resulting in a change in life history strategies (Barton, 2002; Hawlena and Schmitz, 2010). As discussed in the two previous chapters, the threat of predation can also induce changes in behaviours that can result in reduced nutrient uptake (Billman *et al.*, 2011; Clinchy *et al.*, 2013) or food conversion efficiency (Walsh *et al.*, 2016), indirectly affecting allocation to both reproductive effort (Jorgensen *et al.*, 2006; Mukherjee *et al.*, 2014; Grégoir *et al.*, 2018) and brain mass (van der Bijl *et al.*, 2015; Kotrschal *et al.*, 2017; Samuk *et al.*, 2018). Although my thesis suggests that predation risk and drainage drive variation in brain mass and reproductive effort in *B. occidentalis*, these same environmental factors do seem to affect the associations between brain mass and the specific reproductive traits. Despite mounting evidence demonstrating that limited resources, predation risk and other environmental variation can mediate trade-offs in life history traits (Reznick *et al.*, 2001; Schreck, 2010; Travers *et al.*, 2010; Gonda *et al.*, 2012; Heins *et al.*, 2016; Su *et al.*, 2020), my thesis does not provide support that predation risk or drainage are limiting factors that contribute to differences in allocation strategies, at least in terms of reproduction and brain mass variation under the guise of the “Expensive Tissue Hypothesis”. Future studies should investigate other fitness traits such as electric signal production, courtship behaviour, gut size as well as individual brain region variation in order to get a better understanding of how ecological factors contribute to variations in life history strategies of highly encephalized vertebrates.

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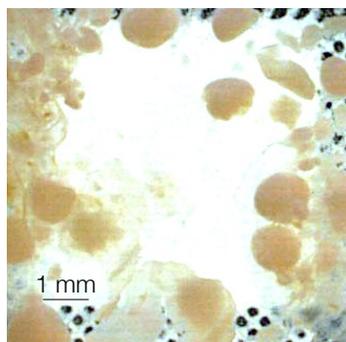
APPENDICES

Appendix 1. Relative predator risk based on incidence of tail injury in *B. occidentalis*.

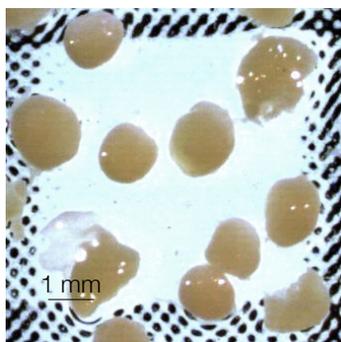
Drainage	Site	Tail Injury	% Tail Damage	Predator Density
Bayano	Tapagrilla	5/22	22.7	High
	Tumagantí	1/14	7.4	Low
Chagres	Frijolito	5/25	20.0	High
	Puente	2/21	9.5	Low

Note: Tail injury encompassed the number of injured fish and the total fish sampled. Fish injury included partial or complete tail loss, as well as observed tail regeneration. Tail damage percentage was determined as the percentage of injured *B. occidentalis* (juveniles and adults) in each population. Predator density studies were conducted by Tran (2014), confirmed by Dunlap *et al.* (2016), by counting number of *R. quelen* caught in transects per hour.

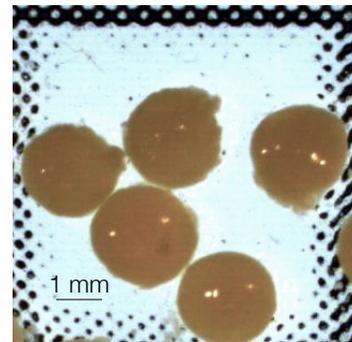
a) Immature oocytes



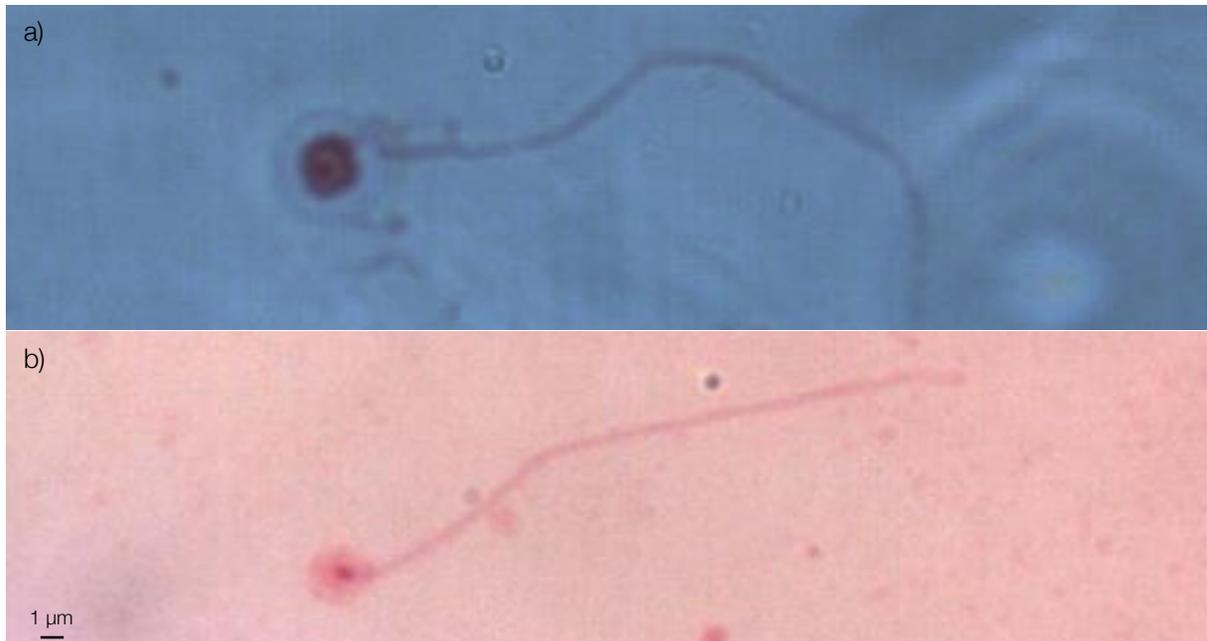
b) Maturing oocytes



c) Mature oocytes



Appendix 2. Oocyte maturity stages in female *B. occidentalis*. a) immature oocytes, b) maturing oocytes and c) mature oocytes. Pictures are from the same Frijolito female (1-046). Average immature oocyte volume was 0.071 mm^3 , average maturing oocyte volume was 0.361 mm^3 and average mature oocyte volume was 1.609 mm^3 .



Appendix 3. Average *B. occidentalis* sperm morphology from a) high predation and b) low predation risk sites (100x). a) Spermatozoa from Frijolito, head width (HW) = 2.14 μm , head length (HL) = 2.15 μm and flagellum length (FL) = 27.42 μm . b) Spermatozoa from Tumagantí, HW = 1.95 μm , HL = 2.03 μm and FL = 20.26 μm .



Appendix 4. Approximate location of the electric organ (clear line) in *B. occidentalis*. Adapted from Salazar *et al.* (2013).